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Melamine Proficiency Test 2009

— Assessing the capabilities of control laboratories to measure melamine in skimmed milk powder and starch-containing foods.

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1. INTRODUCTION

Melamine (2,4,6-triamino-1,3,5-triazine, $C_3H_6N_6$, MW 126.1) is an industrial chemical mostly used in the production of plastics. It contains 67 % nitrogen per mass unit and has an acute toxicity comparable to table salt (sodium chloride). Sometimes melamine is fraudulently added to food and feed products to increase the apparent protein content, as protein concentrations are typically measured by analysis of total nitrogen.

Even though the acute toxicity of melamine is low little is known about its side-products ammeline, ammelide, and cyanuric acid and possible interactions with them. Melamine and cyanuric acid are known to form complexes of low solubility. Intake of melamine has been linked to kidney stones and other health problems.

Late in 2008 a health scare in China concerning melamine tainted powdered milk was near its climax. Although the EU does not import milk or other dairy produce from China processed foods such as biscuits and chocolates might contain milk powder which raised concerns about possible melamine contamination in products on the European market. The European Commission consequently decided that composite products, including feed, that contain milk products originating in or consigned from China shall be checked, including laboratory analysis (Commission Decision 2008/798/EC) [1]. Products containing more than 2.5 mg/kg melamine are to be immediately destroyed.

After a request by the European Commission's Directorate-General for Health and Consumer Protection (DG SANCO) the idea was born to conduct a proficiency test to assess the capabilities of laboratories to determine melamine. Below we describe the different stages and the results of this proficiency test.

2. RATIONALE AND DESIGN

In order to assess the capabilities of laboratories to determine melamine a milk powder and a baking mix, representing starch-containing foods like bread and biscuits, were to be produced and distributed. The milk powder was to be contaminated well above and the baking mix close to the maximum level established in European legislation.

This Proficiency Test was free of charge for participants and carried out according to the IUPAC protocol [2]. Each participant received the two materials as well as an electronic reporting form to report the mass fraction of melamine in the materials, the apparent recovery, an estimation of the measurement uncertainty, and questions about the employed methodology. The target standard deviation for scoring was based on the Horwitz equation [3]:

$$\sigma_p = 0.02 x_a^{0.8495}.$$

This was communicated to the participants in the invitation letter.

The response to a call for registration was so overwhelming that the PT was fully subscribed before the announced end date of registration and registration had to be closed prematurely.

3. PREPARATION OF TEST MATERIALS

3.1. Milk powder stock

Melamine-free skimmed milk powder was obtained from a local wholesaler. One kg of this powder was suspended in 2 L of acetonitrile (ACN)/water (50/50, v/v). After thorough mixing 30 mL of a solution of 1 mg/mL Melamine (courtesy of Agence fédérale pour la Sécurité de la Chaîne alimentaire, Liège, Belgium) in ACN/water (50/50, v/v) were added.

After stirring for one hour the contaminated milk powder suspension was freeze-dried over a weekend. The resulting coarse material was then milled (Retsch ZM200) to a particle size that corresponded to the size of the native milk powder. A determination of the water content (Karl-Fischer) showed comparable water contents for the contaminated freeze-dried material and the native milk powder.

The contaminated milk powder stock (30 mg/kg Melamine) was stored at below -18 ºC until further processing.

3.2. Milk powder PT material

A portion of contaminated milk powder was blended with an equal weight of melamine-free skimmed milk powder. After initial manual mixing the blend was run trough a mill (Retsch ZM200) and then through a spinning divider (Retsch PT100) dividing the initial blend into 10 subsamples. These subsamples were then combined again. The manual mixing, milling, dividing, and combining was repeated twice. The resulting contaminated milk powder was then blended again with an equal weight of melamine-free skimmed milk powder. Starting with the milk powder stock the above process was repeated until a milk powder contaminated at 10 mg/kg melamine was obtained.

The resulting material was filled into two different types of glass vials, either with Teflon-lined screw caps or rubber-lined crimp caps, at ca. 5 g per unit. The two types of glass vials were necessitated by the larger than expected number of participants. The test units were stored at below -18 ºC until further processing.

3.3. Baking mix PT material

Several different melamine-free baking mixes (principal constituents: wheat flour, sugar, dried milk, dried egg) were purchased at local stores. They were combined, mixed and then blended with the contaminated milk powder stock according to the scheme detailed above. The resulting baking mix PT material contained > 10% dried milk by weight and was contaminated at 3 mg/kg melamine.

As above the resulting material was filled into two kinds of glass vials at ca. 5 g per unit and capped with either Teflon-lined screw caps or rubber-lined crimp caps. The test units were stored at below -18 ºC until further processing.

4. HOMOGENEITY OF THE TEST MATERIALS

4.1. Testing method for melamine

A GC-MS method published by the United States Food and Drug Administration was modified for our purposes; in particular, the pre-treatment of the sample was different. In brief, 0.5 g milk powder were dissolved in 10 mL water/diethyl amine (80/20, v/v). After complete solvation 10 mL ACN were added. For the baking mix 0.5 g were suspended in 5 mL ACN/water/diethyl amine (50/40/10, v/v/v) and heated for 11 min in a water bath at 95 ºC. After cooling to room temperature in a cold water bath the volume was made up to 20 mL with fresh extraction solvent.

The so treated milk powder or baking mix samples were then sonicated for 30 min and shaken for another 30 min. After spinning down particulate matter in a centrifuge 200 µL supernatant were transferred into a centrifuge tube containing 30 ng of labelled melamine (2,4,6-$^{15}N_3$-triamino-1,3,5-$^{13}C_3$-triazine, Cambridge Isotope Laboratories). 400 µL ACN were added and precipitated protein was spun down again.

The supernatant was transferred into a deactivated screw-cap vial and evaporated to dryness under a gentle stream of nitrogen. The dry extract was dissolved and derivatised with 100 µL N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA)/ trimethylchlorosilane (TMCS) (99/1, v/v) for 15 min at 80 ºC to yield melamine-tris-TMS. MSTFA was purchased from Pierce, Thermo Scientific, Belgium, and TMCS from Fluka, Sigma-Aldrich, Belgium. 1 µL of this solution was injected into the GC-MS.

GC conditions: DB5-MS 30m x 0.25 mm I.D, 0.25 µm film; split 1:10; temperature program: 120 ºC, 30 ºC/min, 180 ºC, 5 ºC/min, 215 ºC, 30 ºC/min, 300 ºC, 1 min; injector 250 ºC; transfer line 280 ºC.

MS conditions: single ion monitoring mode, ions m/z 327 & 342 for melamine-tris-TMS, ions m/z 333 & 348 for $^{13}C_3$-$^{15}N_3$-melamine-tris-TMS.

Relative repeatability standard deviations were 3 % and 5 % for milk powder and baking mix, respectively.

4.2. Verification of sufficient homogeneity

To verify homogeneity of the PT material 10% of the total number of units per material were picked at random, e.g. 13 baking mix units and 13 milk powder units. Two independent determinations were performed per unit. Sufficient homogeneity was assumed if the between-unit variance (s^2_{sam}) was smaller than some critical value c ([2], Sec. 3.11.2):

$$s^2_{sam} < c$$

$$c = 1.75 \times \sigma^2_{all} + 0.8 \times s^2_{an}$$

The between-unit variance (s^2_{sam}) and the within-unit variance (s^2_{an}) were obtained from one-way analysis of variance (ANOVA). The allowable variance (σ^2_{all}) was calculated as $(0.3*\sigma_p)^2$ from the target standard deviation

(σ_p) of 1.12 mg/kg for milk powder and 0.43 mg/kg for baking mix. Table 1 shows details.

		Milk powder	Baking mix
Critical value	c	0.272	0.044
Between-unit variance	s^2_{sam}	0.0188	0.004
Within-unit variance	s^2_{an}	0.0933	0.0195
Allowable variance	σ^2_{all}	0.113	0.0165

Table 1: Results of homogeneity testing; all values are in mg/kg.

For both materials, milk powder and baking mix, s^2_{sam} was smaller than c and therefore sufficient homogeneity was assumed.

5. DETERMINATION OF THE ASSIGNED VALUE

To determine assigned values for the milk powder and the baking mix materials, traceable to SI units and with small measurement uncertainties, exact-matching double isotope dilution mass spectrometry (IDMS) [3] was employed. To that end blends of the test materials and isotopically labelled melamine (ISTD) (sample blend, SB) and of a melamine-free matched material, a melamine reference solution, and ISTD (calibration blend, CB) were prepared gravimetrically and measured by GC-MS.

5.1. Preparation of blends

Three units each of the milk powder and the baking mix PT material were selected randomly. Of each unit two sample blends were prepared for a total of six independent preparations for each material.

The six SBs of milk powder were prepared by weighing between 0.499 g and 0.501 of the test material into 50 mL conical polypropylene centrifuge screw-cap tubes on an analytical balance (Sartorius ME235S, d=0.01 mg). After addition of 20 mL of water/ formic acid (99/1, v/v) the blends were spiked gravimetrically with a solution of 10 µg/mL isotopically labelled melamine (2,4,6-$^{15}N_3$-triamino-1,3,5-$^{13}C_3$-triazine, Cambridge Isotope Laboratories) in water (ISTD).

To avoid weighing errors due to evaporative loss of solvent during the weighing procedure a syringe of appropriate size was filled with the spiking solution, capped, and weighed. Then solution was dispensed into the blend and the syringe capped, and weighed again. The difference between the weight of the full syringe and the weight of the syringe after dispensing was taken as the mass of solution added. Between 0.530 g and 0.545 g of 10 µg/mL ISTD were added this way.

The weights for the six baking mix SBs ranged from 0.500 g to 0.501 g for the PT material and 0.173 g to 0.182 g for the ISTD.

Calibration blends (CB) were analogously prepared from either a solution of 10.0 µg/g melamine (gravimetrically prepared, melamine purity > 99%, courtesy of Agence fédérale pour la Sécurité de la Chaîne alimentaire, Liège, Belgium) in ACN/water (50/50, v/v, REF1) or of a commercial, certified solution of 103.8 µg/mL melamine (Biopure, Tulln, Austria) diluted to 12.1 µg/g in ACN/water (84/16, v/v, REF2).

For each of the two proficiency test materials and each of the two melamine reference solutions two CBs were prepared by weighing 0.5 g of a melamine-free matched material into eight 50 mL conical polypropylene centrifuge screw-cap tubes. After addition of the 20 mL extraction solvent (water/ formic acid, 99/1, v/v) the ISTD and REF1 or REF2 were weighed in as described before. Table 2 lists the weights for the eight CBs.

	Preparation 1		**Preparation 2**	
	ISTD	**REF1**	**ISTD**	**REF1**
Milk powder	0.559	0.494	0.568	0.495
Baking mix	0.180	0.151	0.183	0.152
	ISTD	**REF2**	**ISTD**	**REF2**
Milk powder	0.573	0.406	0.576	0.407
Baking mix	0.180	0.180	0.183	0.180

Table 2: Weights in g added to calibration blends (CB).

5.2. Extraction and clean-up

While for homogeneity testing a method without sample clean-up was sufficient for the purpose of assigning a reference value a clean-up was necessary to improve precision by eliminating matrix interferences. Therefore a strong cation exchange chromatography clean-up step was introduced which necessitated water/formic acid (99/1, v/v) as extraction solvent. The procedure was as follows:

After preparing them the blends were sonicated for 10 min and then shaken on a horizontal shaker for another 10 min. There was no heating step for the baking mix as in the homogeneity testing method since it led to losses of the labelled melamine. Then particulate matter was spun down for 10 min at a relative centrifugal force of 3200 *g*. Strong cation exchange SPE cartridges

(Discovery DSC-SCX 3mL/500mg, Supelco) were conditioned with 3 mL methanol and 3 mL water using a vacuum manifold. One mL of the supernatant after centrifugation was applied followed by washing with 1 mL water and 1 mL methanol/ 25% ammonia (90/10, v/v). The melamine fractions were eluted with six times 0.5 mL methanol/diethyl amine (95/5, v/v) into deactivated tubes. The eluates were taken to dryness under a gentle stream of nitrogen.

5.3. Measurement

The dry extracts were dissolved and derivatised to the tris-TMS derivatives with 100 µL MSTFA/ TMCS (99/1, v/v) for 15 min at 80 ºC. 1 µL of these solutions were injected into the GC-MS.

GC conditions: DB5-MS 30m x 0.25 mm I.D, 0.25 µm film; pulsed splitless injection, pulse time 1 min; temperature program: 120 ºC, 1 min, 30 ºC/min, 180 ºC, 5 ºC/min, 215 ºC, 30 ºC/min, 300 ºC, 1 min; injector 250 ºC; transfer line 280 ºC

MS conditions: single ion monitoring mode, ions m/z 327 & 342 for melamine-tris-TMS, ions m/z 333 & 348 for $^{13}C_3$-$^{15}N_3$-melamine-tris-TMS, dwell times 10 ms all.

Injection sequences were made up of a calibration blend run followed by a sample blend run. This was repeated until a total of 5 runs for each blend were obtained.

5.4. The assigned values and their uncertainties

The aim of exact-matching double IDMS is to obtain ion ratios of native analyte to labelled analyte of close to one in the sample and calibration blends. Furthermore the ratios of the sample blend ion ratio to the calibration blend ion ratio should also be close to one. By observing this instrumental bias becomes negligible and direct traceability to SI units is given.

The ions m/z 327 ($[M-15]^{+\cdot}$, melamine-tris-TMS) and m/z 333 ($[M-15]^{+\cdot}$, $^{13}C_3$-$^{15}N_3$-melamine-tris-TMS) were chosen for the calculation of the ion ratios. Since the contribution of the labelled melamine to m/z 327 was negligible, and the contribution of melamine to m/z 333 was not detectable, the following model equation was used:

$$w_{s,i} = w_{c,i} \times \frac{m_{c,i} A_{ISTD,CB}}{m_{ISTD,CB} A_{Mel,CB}} \times \frac{m_{ISTD,SB} A_{Mel,SB}}{m_{smp,i} A_{ISTD,SB}}$$

with

$w_{s,i}$ = mass fraction of melamine in test portion

$w_{c,i}$ = mass fraction of melamine in the reference solution

$m_{c,i}$ = mass of the melamine reference solution

$m_{ISTD,CB}$ = mass of the ISTD solution added to CB

$m_{ISTD,SB}$ = mass of the ISTD solution added to SB

$m_{smp,i}$ = mass of test portion

$A_{Mel,SB}$ = Peak area melamine m/z 327 in SB

$A_{ISTD,SB}$ = Peak area labelled melamine m/z 333 in SB

$A_{Mel,CB}$ = Peak area melamine m/z 327 in CB

$A_{ISTD,CB}$ = Peak area labelled melamine m/z 333 in CB

For each corresponding pair of a CB run and a SB run $w_{s,i}$ was calculated as above. The assigned value was calculated as the average of all $w_{s,i}$ per material:

$$x_a = \overline{w}_{s,i} \times F_{\Pr ec}$$

The uncertainty of x_a is then given by:

$$u(x_a) = x_a \times \sqrt{\frac{\sum u^2(w_{s,i})}{n x_a^2} + \frac{u^2(F_{\Pr ec})}{x_a^2}}$$

The factor F_{Prec} has a value of one and has been added to account for uncertainties due to intermediate precision combining the repeatability effects of all terms in the model equation. Uncertainties of $w_{s,i}$ ($u(w_{s,i})$) are due to the preparation and purity of the two reference solutions and the bias of the mass determinations. The following factors were included in the determination of the intermediate precision: two different reference solutions and two different materials on two days with two operators. Table 3 lists the results:

	Assigned value (x_a) **[mg/kg]**	**Expanded Uncertainty ($U(x_a)$)** **[mg/kg]**	**Coverage factor (k)**
Milk powder	10.0	0.6	2
Baking mix	3.18	0.17	2

Table 3: Assigned values and expanded uncertainties

The principal contributors to the combined uncertainties of both materials were the intermediate precision (97%, relative uncertainty $u_r = 0.026$) and the uncertainty of the reference solutions (3%, $u_r = 0.003$). The uncertainties due to mass determinations were negligible.

6. RESULTS

Laboratories of 31 countries, including 21 of the 27 Member States of the European Union, reported back 114 results for the milk powder and 112 for the baking mix (see Annex A for details).

6.1. Mass fraction of Melamine

In the reporting form it was not requested that results were to be reported corrected for recovery. However, some laboratories found it unclear whether or not to correct for recovery. In the evaluation we assumed all reported values to be uncorrected for recovery, unless a labelled internal standard was used (implicit correction), the use of standard addition was remarked (implicit correction), no recovery was reported, or it was remarked that correction was already included.

For each result a z-score was calculated as follows:

$$z = \frac{x - x_a}{\sigma_p}$$

with x being the individual result, x_a the assigned value (5.4) and σ_p the target standard deviation:

Milk powder: $\sigma_p = 1.12$ mg/kg

Baking mix: $\sigma_p = 0.43$ mg/kg

Figure 1 and Figure 2 depict individual results in ascending order for all laboratories. It can be seen that practically three-quarters of all results for the milk powder (74%) and the baking mix (73%) were satisfactory ($-2 \leq z \leq 2$). Individual z-scores are listed in Annex B.

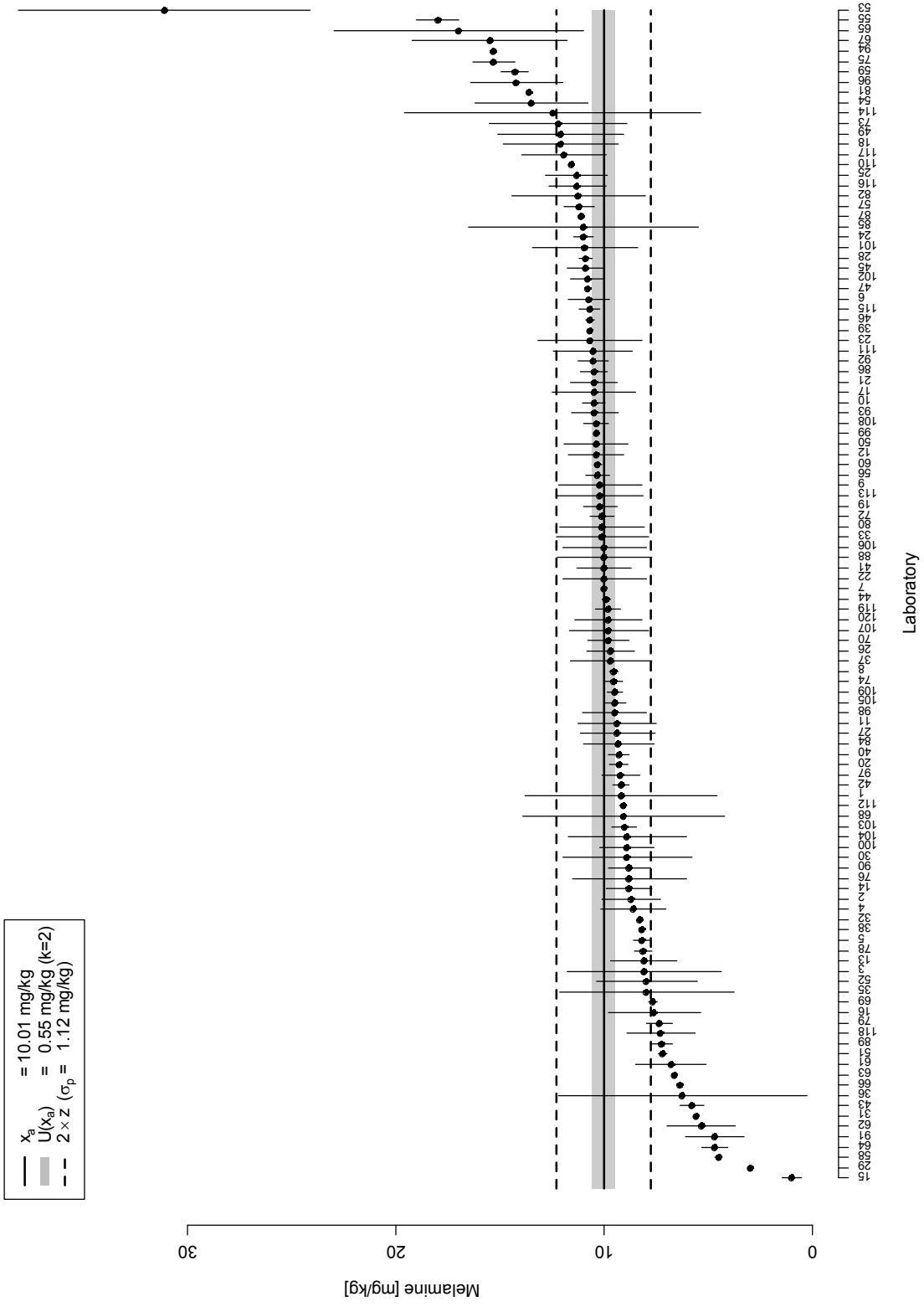

Figure 1: Individual results for milk powder with their measurement uncertainty ranges; solid line and grey band depict the assigned value with its expanded uncertainty; broken lines depict the satisfactory performance range

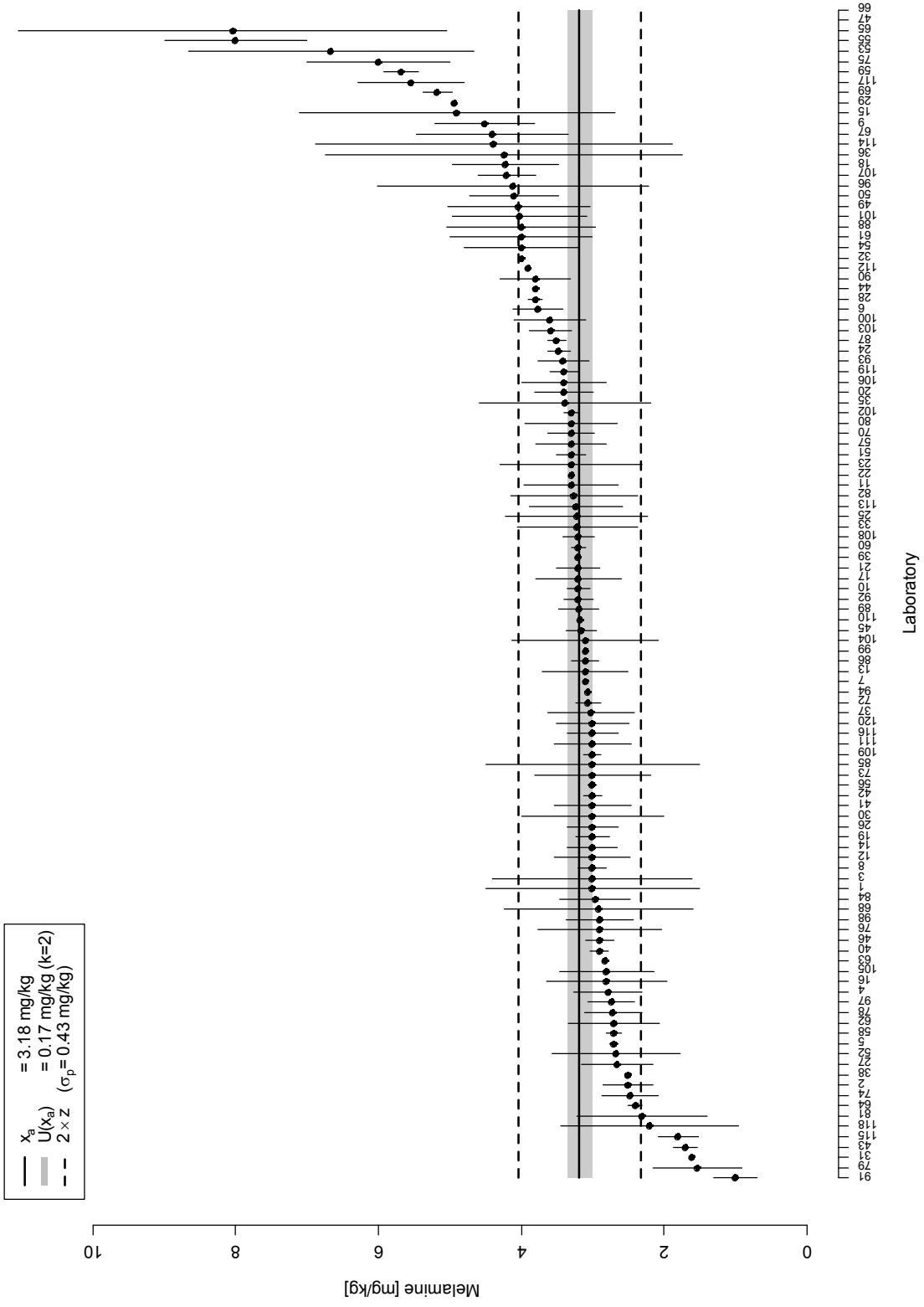

Figure 2: Individual results for baking mix with their measurement uncertainty ranges; solid line and grey band depict the assigned value with its expanded uncertainty; broken lines depict the satisfactory performance range

6.2. Measurement uncertainties (MU)

According to the "Report on the Relationship between Analytical Results, Measurement Uncertainty, Recovery Factors and the Provisions of EU Food and Feed Legislation" [4] the uncertainty of a measurement result is a prerequisite for decisions as to whether a sample or batch from which a sample is taken is in compliance with relevant EU legislation. Therefore the participating laboratories were asked to report an estimate of their MU and for 90% of the reported results that was done.

Comparing the individual results plus/minus their associated MUs with the assigned values plus/minus their associated MUs enables one to determine whether participants properly estimated their MU. Proper estimation would mean there is an overlap. When there is no overlap MU was clearly underestimated.

This situation is depicted in Figure 3 and Figure 4. Of the reported MUs 26 (23%) were underestimated for the milk powder and 25 (22%) for the baking mix. One reason for underestimation could be that biases of the analysis method were not properly accounted for. In case of compliance testing underestimation of MU might have serious consequences and the affected laboratories should verify their uncertainty budgets.

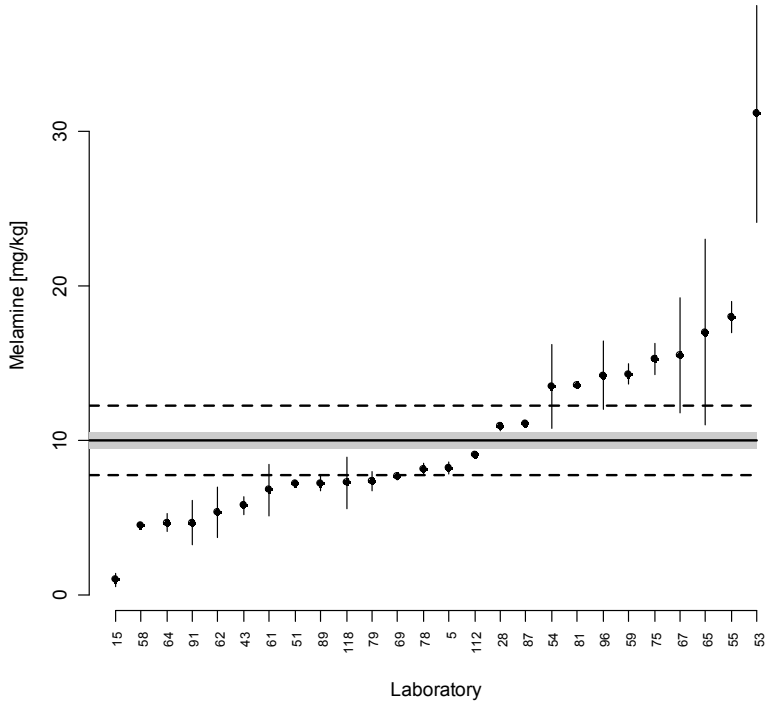

Figure 3: Milk powder results for which measurement uncertainty was under estimated

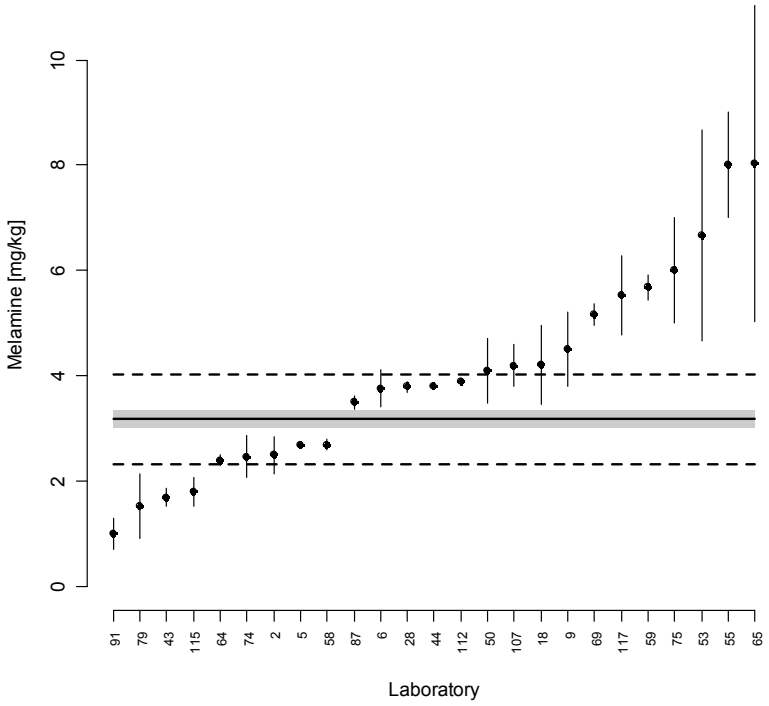

Figure 4: Baking mix results for which measurement uncertainty was under estimated

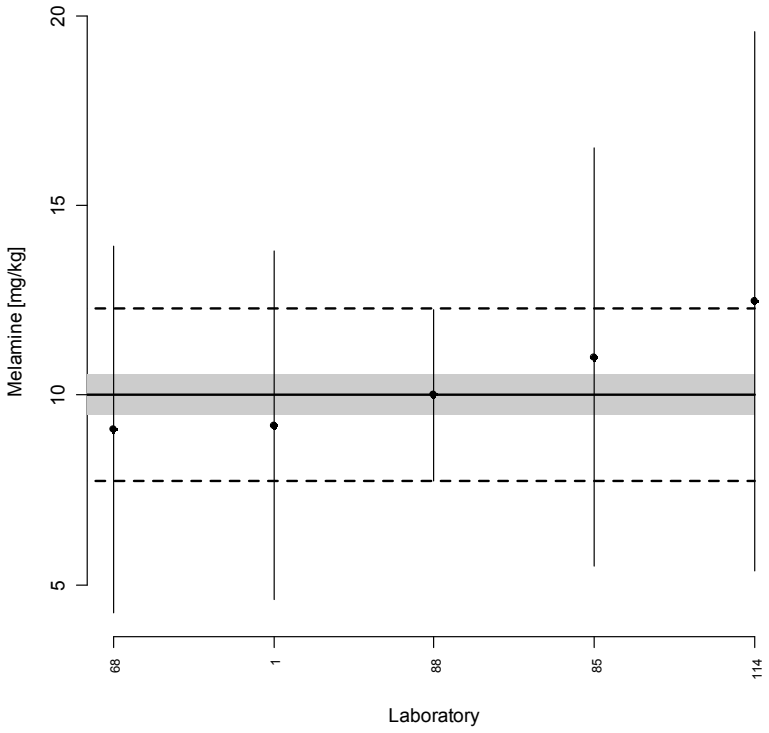

Figure 5: Milk powder results for which measurement uncertainty estimation was rather conservative

Figure 5 and Figure 6 show results for which MU was estimated rather conservatively. This was the case for 5 (4%) milk powder results and 11 (10%) baking mix results. While such conservative estimation of MU has not the same consequences as underestimation clients will more likely than not choose laboratories with narrower MUs.

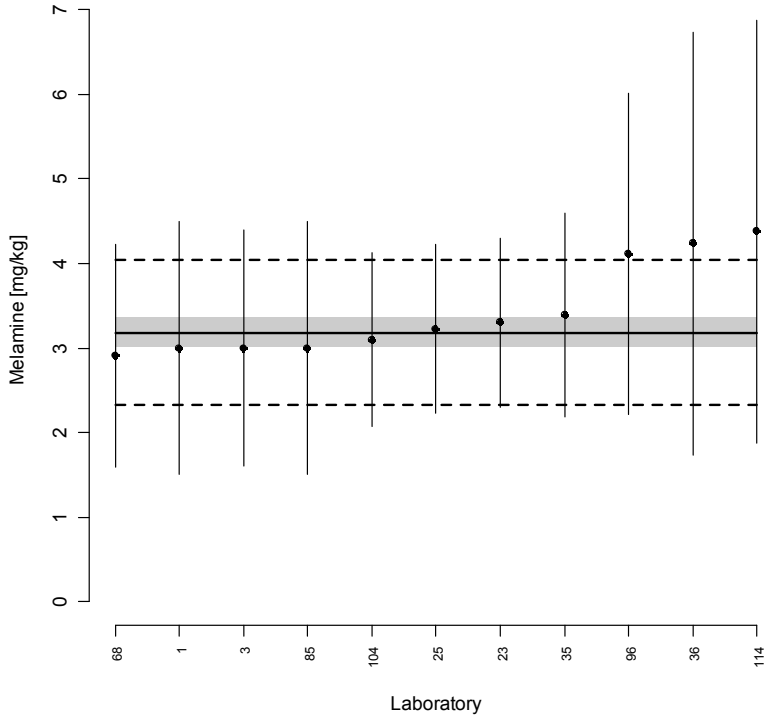

Figure 6: Baking mix results for which measurement uncertainty estimation was rather conservative

The remaining results, 71 (62%) for the milk powder and 65 (58%) for the baking mix, had associated MU ranges which overlapped with the assigned value and its MU range (Figure 7 and Figure 8) and appeared to be reasonable.

Another way for participants to assess the validity of their MU estimation is to compare their zeta-score:

$$\zeta = \frac{x - x_a}{\sqrt{u^2(x) + u^2(x_a)}}$$

with their z-score. Instead of a target standard deviation the zeta-score uses the combined uncertainties of a participant's reported result and the material's assigned value as the denominator. Here a high absolute score could be due to underestimation of one's own MU and/or gross error in the

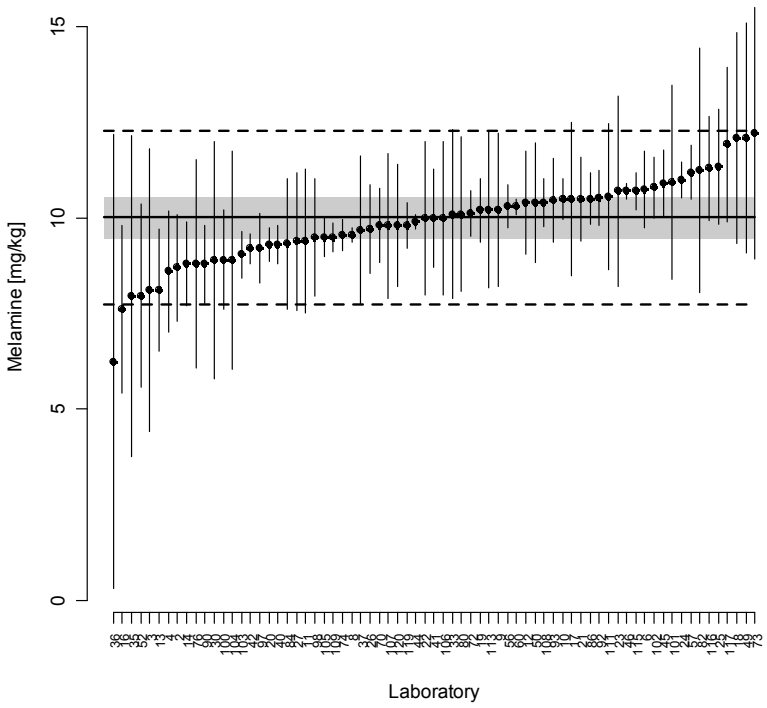

Figure 7: Milk powder results for which measurement uncertainty was properly estimated

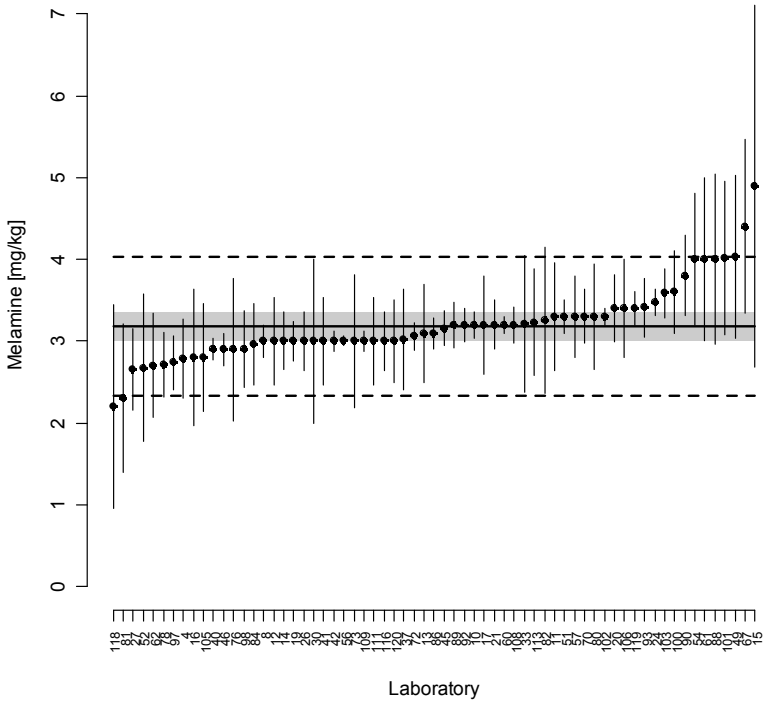

Figure 8: Baking mix results for which measurement uncertainty was properly estimated

determination of the content. Vice versa, a low score could be due to a too conservative MU estimate. Therefore zeta-scores should always be considered together with z-scores. Annex B lists, next to the z-scores, the individual zeta-scores for all reported results.

6.3. Method parameters and influences on results

All reports included details about the employed methodology (see Annex C). A few of the reported method parameters were examined for their potential influence on the results.

Whether an ***internal standard*** (ISTD) was used and, if yes, which one was one question. The majority of all results (49, 43%) were acquired with stable-isotope labelled melamine, 38 (33%) without the use of ISTD, and 27 (24%) with some other ISTD. The use of labelled melamine as ISTD which may be called isotope dilution mass spectrometry (IDMS) in the wider sense had definitely a positive effect on results.

While the mean of the group with IDMS was not much different from the group using other or no ISTD the standard deviation was. Figure 9 shows clearly that the spread of results from milk powder is much smaller if using IDMS. The same was true for the baking mix (not shown). This had also an effect on the z-scores: almost 90 % of the results for milk powder and baking mix obtained with IDMS were satisfactory. Without IDMS that dropped to around 60 %.

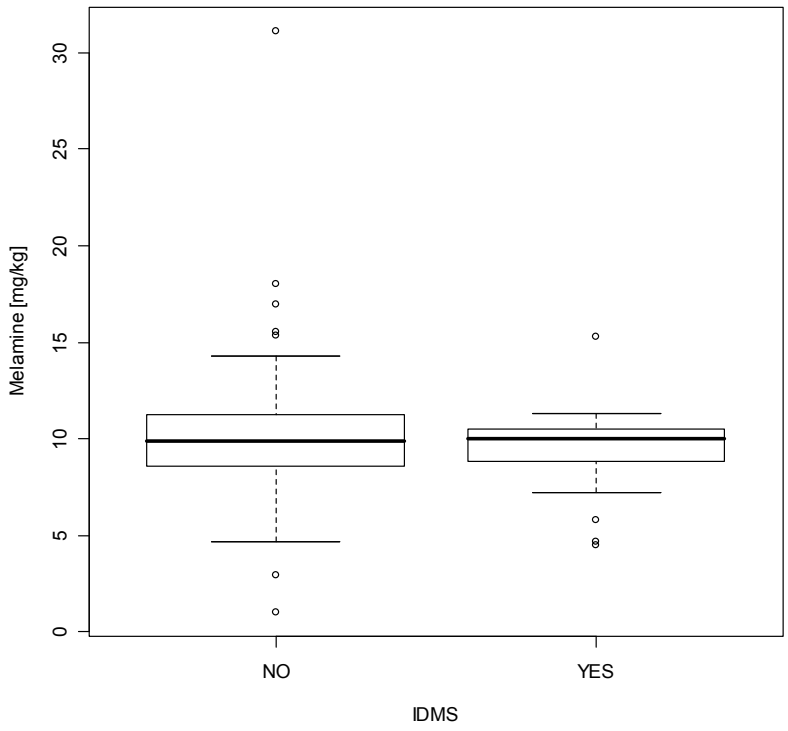

Figure 9: Effect of use of stable-isotope labelled ISTD (isotope dilution mass spectrometry (IDMS); the figure shows a box & whiskers plot of the results of milk powder but effects for baking mix are similar.

Another possibly influential parameter is the ***extraction solvent***. Here a wide variety of different solvent mixes have been used. The mostly used extraction solvent was "acetonitrile/water (50/50)" (in 32 (28%) cases), followed by "acetonitrile/water/diethyl amine (50/40/10)" in 26 (23%), "2.5 % formic acid" in 6 (5%), "acetonitrile/water/1 N HCl (48/48/4)" in 5 (4%), and a multitude of others which were used in just one or two methods at a time.

A further question asked was whether a ***clean-up*** was used. We divided the answers into three groups based on whether an unspecified, a strong cation exchange (SCX), or no SPE clean-up was carried out. The majority of all results, 66 of 114 (58%), were obtained without a SPE clean-up. This group combined reports with no answer to this question, those that reported removal of particulate matter by centrifugation and/or filtration, and those which removed protein by precipitation. Of the remaining reports 41 (36%) named SCX and 7 (6%) other SPE as clean-up.

The ***instrumentation*** used to determine melamine was of interest, too. Of the 114 reported results the majority (70 or 61%) were acquired with LC/MS(/MS), 30 (26%) with GC/MS, 8 (7%) with HPLC/UV, 5 (4%) with enzym-linked immunosorbent assays (ELISA), and one (1%) with capillary electrophoresis/UV.

These three parameters, extraction solvent, clean-up, instrumentation, can be grouped into several distinct groups. There are five groups with a large enough population for a statistical evaluation. Those groups are listed in Table 4.

Group	N	Separation	Extraction solvent	Clean-up with SPE	Use of IDMS by:
1	23	GC	Acetonitrile/Water/Diethyl amine (50/40/10)	NO	5
2	13	LC	Acetonitrile/Water (50/50)	NO	6
3	13	LC	Acetonitrile/Water (50/50)	SCX	9
4	5	LC	2.5 % formic acid	NO	1
5	5	ELISA	varying	NO	0

Table 4: Parameters of the five groups with the largest numbers of members

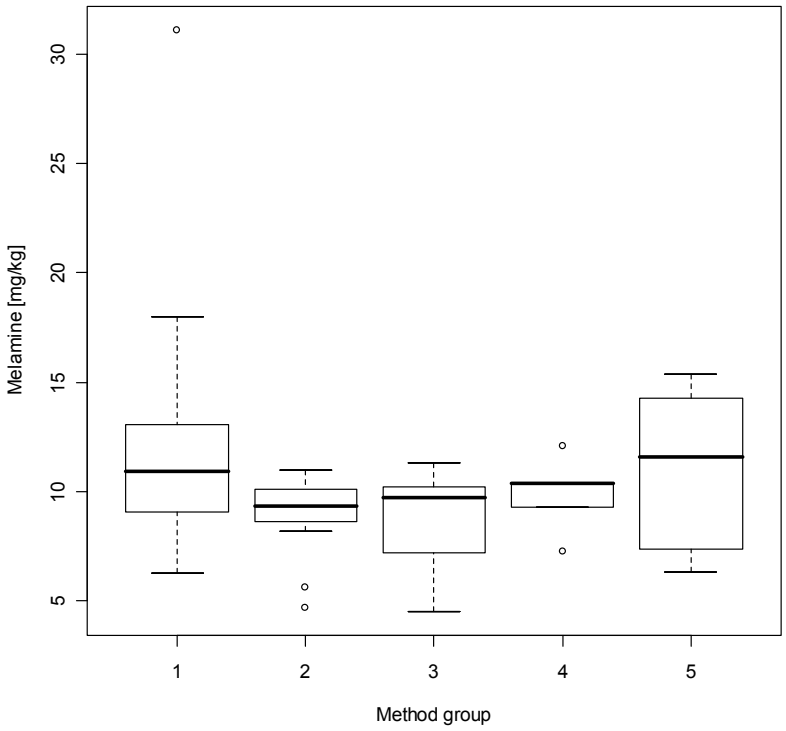

Figure 10: Effects of the five parameter groups on the results of the milk powder

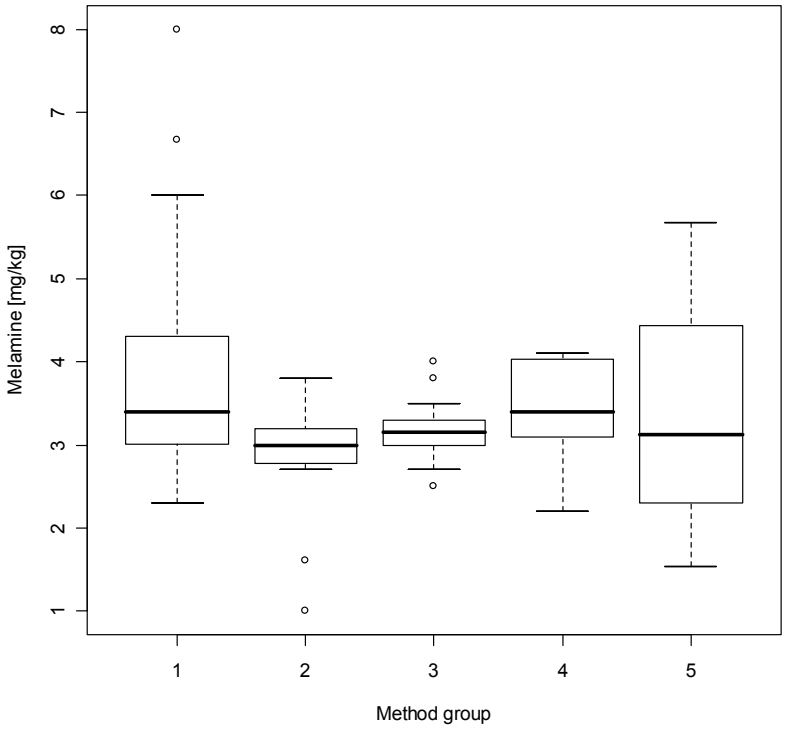

Figure 11: Effects of the five parameter groups on the results of the baking mix

Parameter groups 1 and 3 resemble methods published by the United States Food and Drug Administration (FDA) [5]. Box & Whisker plots of the effects of the different groups on the results of milk powder and baking mix are displayed in Figure 10 and Figure 11. Group medians were not significantly different; in particular, groups 2 and 3 which differ only by whether an SCX clean-up was used perform comparably. The differences in spread of results are related to the use of IDMS.

7. CONCLUSIONS

The main outcome of this proficiency test was that of the 114 reported results for the milk powder 74% were satisfactory. Of the 112 reported results for the baking mix 73% were satisfactory.

Next to this assessment of the capabilities to determine melamine in two different materials the ability to properly estimate measurement uncertainty was assessed. 90% of all reported results were accompanied by a measurement uncertainty (MU) and the majority of the results (62% milk powder, 58% baking mix) were reported with a reasonable MU range. For 23% of the milk powder and 22% of the baking mix results MU was underestimated. Since such underestimation may have serious consequences in compliance testing the affected laboratories are advised to reconsider their uncertainty budgets. The remaining small number of the MUs was estimated rather conservatively.

Considering the reported method parameters it can be said that the use of isotope dilution mass spectrometry with a stable-isotope labelled melamine is clearly advantageous with regards to the accuracy of the result. Comparing different method parameter groups, of which two resemble methods published by the United States Food and Drug Administration, there is none which would outperform others.

8. ACKNOWLEDGEMENTS

We want to thank Carsten Mischke for his valuable technical assistance in preparing the proficiency test materials and Anne-Mette Jensen for reviewing and improving the manuscript.

9. REFERENCES

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10. ANNEX A

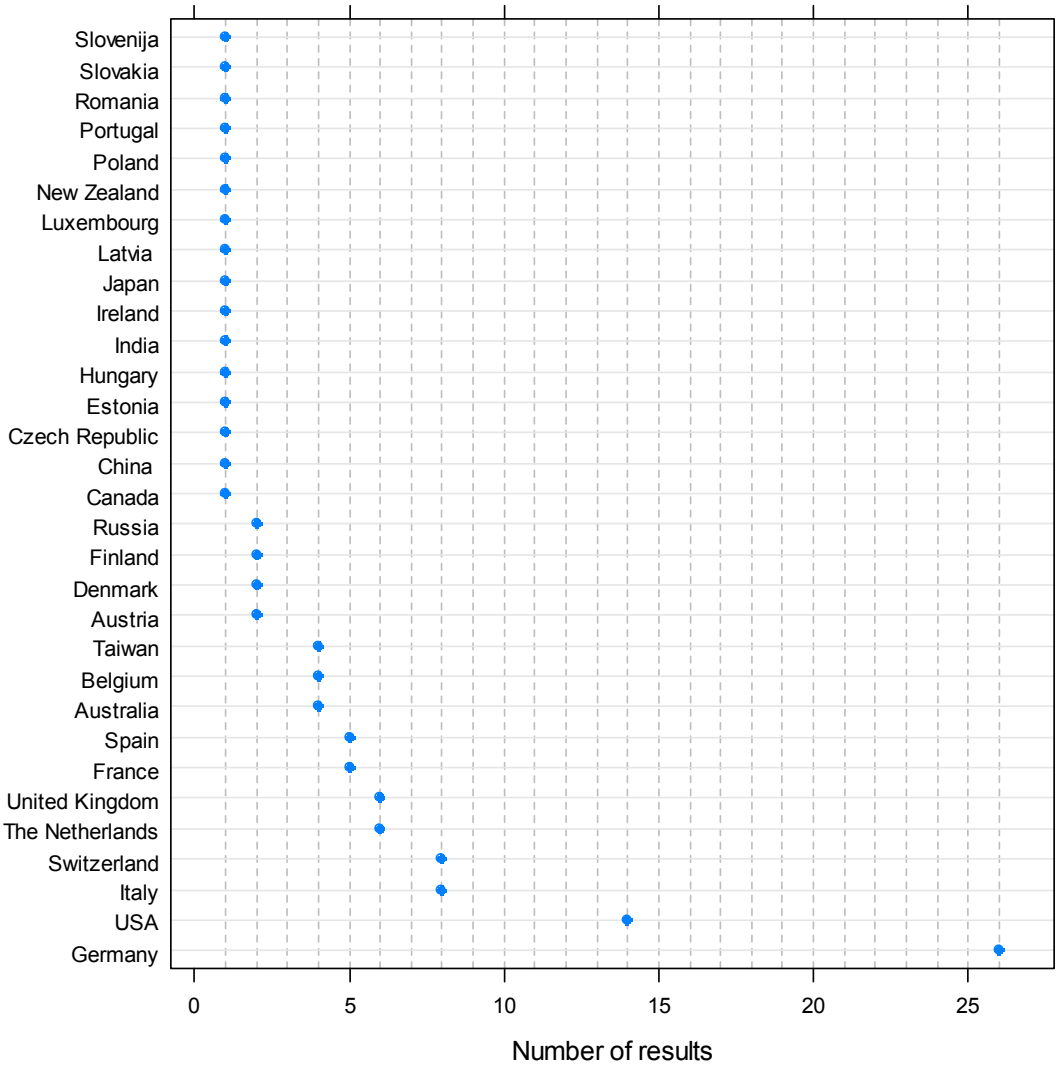

Figure A. 1: Number of results for milk powder reported per country; for baking mix the Portuguese and one laboratory of the United Kingdom did not report back a result.

11. ANNEX B

Table B 1: Reported result, assumption for recovery correction, value used for calculations, reported recovery, reported expanded measurement uncertainty, calculated z-score, and calculated zeta-score for the milk powder by laboratory

Laboratory ID	Mass fraction reported [mg/kg]	Mass fraction assumed corrected for recovery	Mass fraction used [mg/kg]	Reported recovery [%]	Reported MU (k=2) [mg/kg]	z-score	zeta-score
1	9.2	YES	9.2	125	4.6	-0.7	-0.3
2	8.7	YES	8.7	100	1.39	-1.2	-1.8
3	8.1	YES	8.1	99	3.7	-1.7	-1.0
4	10.5	NO	8.6	122	1.58	-1.3	-1.7
5	8.2	YES	8.2	100	0.39	-1.6	-5.4
6	10.0	NO	10.8	93	1	0.7	1.3
7	10.0	YES	10.0	75		0.0	
8	8.6	NO	9.6	90	0.2	-0.4	-1.6
9	9.9	NO	10.2	97	2	0.2	0.2
10	10.5	YES	10.5	103	0.53	0.4	1.3
11	9.4	YES	9.4	125	1.88	-0.5	-0.6
12	10.4	YES	10.4	98	1.35	0.3	0.5
13	8.1	YES	8.1	100	1.6	-1.7	-2.3
14	8.8	YES	8.8	105	1.1	-1.1	-2.0
15	1.0	YES	1.0	87	0.45	-8.0	-25.4
16	7.6	YES	7.6	64	2.2	-2.2	-2.1
17	10.5	YES	10.5	83	2	0.4	0.5
18	11.0	NO	12.1	91	2.76	1.9	1.5
19	10.2	YES	10.2	72	0.82	0.2	0.4
20	9.3	YES	9.3	100	0.43	-0.6	-2.0
21	10.5	YES	10.5	92	1.1	0.4	0.8
22	10.0	YES	10.0	110	2	0.0	0.0
23	10.7	YES	10.7	84	2.5	0.6	0.5
24	11.0	YES	11.0	113	0.47	0.9	2.7
25	10.2	NO	11.3	90	1.5	1.2	1.7
26	9.7	YES	9.7	97	1.16	-0.3	-0.5
27	9.1	NO	9.4	97	1.8	-0.6	-0.7
28	10.9	YES	10.9	90	0.3	0.8	2.8
29	2.7	NO	3.0	91		-6.3	
30	8.9	YES	8.9	88	3.1	-1.0	-0.7
31	5.2	NO	5.6	93		-3.9	
32	8.3	YES	8.3	110		-1.5	
33	10.1	YES	10.1	100	2.2	0.1	0.1

Laboratory ID	Mass fraction reported [mg/kg]	Mass fraction assumed corrected for recovery	Mass fraction used [mg/kg]	Reported recovery [%]	Reported MU (k=2) [mg/kg]	z-score	zeta-score
35	7.0	NO	8.0	88	4.2	-1.8	-1.0
36	9.3	NO	6.2	149	5.95	-3.4	-1.3
37	9.3	NO	9.7	96	1.95	-0.3	-0.3
38	8.2	YES	8.2	70		-1.6	
39	10.7	YES	10.7	102		0.6	
40	9.3	YES	9.3	118	0.51	-0.6	-1.9
41	10.0	YES	10.0	100	1.29	0.0	0.0
42	9.2	YES	9.2	103	0.4	-0.7	-2.4
43	5.8	YES	5.8	98	0.58	-3.8	-10.5
44	10.4	NO	9.9	105	0.19	-0.1	-0.4
45	8.5	NO	10.9	78	0.87	0.8	1.7
46	10.7	YES	10.7	95	0.2	0.6	2.4
47	9.7	NO	10.8	90		0.7	
49	12.0	NO	12.1	99.2	3	1.9	1.4
50	10.4	YES	10.4	90	1.56	0.3	0.5
51	7.2	YES	7.2	76	0.2	-2.5	-9.6
52	7.4	NO	8.0	93	2.4	-1.8	-1.7
53	28.0	NO	31.1	90	7	18.8	6.0
54	13.5	YES	13.5	55	2.7	3.1	2.5
55	9.0	NO	18.0	50	1	7.1	14.0
56	10.3	YES	10.3	103	0.57	0.3	0.7
57	11.2	YES	11.2	98	0.72	1.1	2.6
58	4.5	YES	4.5	66	0.18	-4.9	-19.0
59	13.0	NO	14.3	91	0.65	3.8	10.0
60	10.3	YES	10.3	78	0.2	0.3	1.0
61	3.4	NO	6.8	50	1.7	-2.9	-3.6
62	5.4	NO	5.3	101	1.62	-4.2	-5.5
63	4.3	NO	6.6	65		-3.0	
64	4.7	YES	4.7	185	0.6	-4.7	-13.0
65	16.3	NO	17.0	96	6	6.2	2.3
66	8.0	NO	6.3	126		-3.3	
67	15.5	YES	15.5	87	3.72	4.9	2.9
68	11.0	NO	9.1	121	4.84	-0.8	-0.4
69	9.6	NO	7.7	125	0.2	-2.1	-8.0
70	9.8	YES	9.8	97	0.98	-0.2	-0.4
72	8.6	NO	10.1	85	0.59	0.1	0.3
73	11.0	NO	12.2	90	3.3	2.0	1.3
74	8.5	NO	9.6	89	0.4	-0.4	-1.4
75	15.3	YES	15.3	120	1	4.7	9.3
76	8.8	YES	8.8	74	2.74	-1.1	-0.9

Laboratory ID	Mass fraction reported [mg/kg]	Mass fraction assumed corrected for recovery	Mass fraction used [mg/kg]	Reported recovery [%]	Reported MU (k=2) [mg/kg]	z-score	zeta-score
78	4.8	NO	8.1	59	0.4	-1.7	-5.5
79	6.1	NO	7.3	83	0.62	-2.4	-6.4
80	10.1	NO	10.1	100	2.03	0.1	0.1
81	13.6	YES	13.6	85	0.16	3.2	12.5
82	10.0	NO	11.2	89	3.2	1.1	0.8
84	11.0	NO	9.3	118	1.7	-0.6	-0.8
85	11.0	YES	11.0	90	5.5	0.9	0.4
86	10.5	YES	10.5	81	0.67	0.4	1.1
87	11.1	YES	11.1	103	0.13	1.0	3.9
88	10.0	YES	10.0	70	2.26	0.0	0.0
89	6.8	NO	7.2	94	0.51	-2.5	-7.4
90	8.8	YES	8.8	89	1.02	-1.1	-2.1
91	4.7	YES	4.7	99	1.41	-4.7	-7.0
92	10.2	NO	10.5	97	0.72	0.5	1.1
93	9.2	NO	10.5	88	1.1	0.4	0.7
94	13.5	NO	15.3	88		4.8	
96	12.5	NO	14.2	88	2.2	3.7	3.7
97	8.2	NO	9.2	89	0.92	-0.7	-1.5
98	9.5	YES	9.5	94	1.54	-0.5	-0.6
99	10.4	YES	10.4	90		0.3	
100	8.9	NO	8.9	100	1.3	-1.0	-1.6
101	10.6	NO	10.9	97	2.54	0.8	0.7
102	10.8	YES	10.8	92	0.8	0.7	1.6
103	7.5	NO	9.0	83	0.6	-0.9	-2.4
104	8.9	YES	8.9	100	2.85	-1.0	-0.8
105	9.5	YES	9.5		0.51	-0.5	-1.4
106	10.0	YES	10.0	106	2	0.0	0.0
107	9.8	YES	9.8		1.9	-0.2	-0.2
108	10.4	YES	10.4	102	0.62	0.3	0.9
109	9.5	YES	9.5	97	0.38	-0.5	-1.5
110	11.8	NO	11.6	102		1.4	
111	9.5	NO	10.6	90	1.9	0.5	0.6
112	9.0	NO	9.1	99	0.18	-0.8	-3.2
113	10.2	YES	10.2	104	2.04	0.2	0.2
114	13.1	NO	12.5	105	7.11	2.2	0.7
115	10.7	YES	10.7	88	0.49	0.6	1.9
116	11.3	YES	11.3	89	1.37	1.2	1.7
117	12.4	NO	11.9	104	2.02	1.7	1.8
118	8.3	NO	7.3	114	1.65	-2.4	-3.1
119	10.5	NO	9.8	107	0.6	-0.2	-0.5

Laboratory ID	Mass fraction reported [mg/kg]	Mass fraction assumed corrected for recovery	Mass fraction used [mg/kg]	Reported recovery [%]	Reported MU (k=2) [mg/kg]	z-score	zeta-score
120	9.8	YES	9.8	104	1.6	-0.2	-0.2

Table B 2: Reported result, assumption for recovery correction, value used for calculations, reported recovery, reported expanded measurement uncertainty, calculated z-score, and calculated zeta-score for the baking mix by laboratory

Laboratory ID	Mass fraction reported [mg/kg]	Mass fraction assumed corrected for recovery	Mass fraction used [mg/kg]	Reported recovery [%]	Reported MU (k=2) [mg/kg]	z-score	zeta-score
1	3.0	YES	3.0	125	1.5	-0.4	-0.2
2	2.5	YES	2.5	95	0.35	-1.6	-3.5
3	3.0	YES	3.0	99	1.4	-0.4	-0.3
4	3.2	NO	2.8	115	0.48	-0.9	-1.6
5	2.7	YES	2.7	100	0.06	-1.1	-5.3
6	3.5	NO	3.8	93	0.35	1.4	3.0
7	3.1	YES	3.1	70		-0.2	
8	2.4	NO	3.0	80	0.2	-0.4	-1.4
9	3.7	NO	4.5	82	0.7	3.1	3.7
10	3.2	YES	3.2	103	0.16	0.0	0.2
11	3.3	YES	3.3	130	0.66	0.3	0.4
12	3.0	YES	3.0	107	0.53	-0.4	-0.6
13	3.1	YES	3.1	100	0.6	-0.2	-0.3
14	3.0	YES	3.0	105	0.35	-0.4	-0.9
15	4.9	YES	4.9	87	2.21	4.0	1.6
16	2.8	YES	2.8	88	0.84	-0.9	-0.9
17	3.2	YES	3.2	77	0.6	0.0	0.1
18	3.8	NO	4.2	90	0.75	2.4	2.7
19	3.0	YES	3.0	84	0.24	-0.4	-1.2
20	3.4	YES	3.4	100	0.41	0.5	1.0
21	3.2	YES	3.2	94	0.3	0.0	0.1
22	3.3	YES	3.3			0.3	
23	3.3	YES	3.3	84	1	0.3	0.2
24	3.5	YES	3.5	106	0.16	0.7	2.5
25	2.9	NO	3.2	90	1	0.1	0.1
26	3.0	YES	3.0	97	0.36	-0.4	-0.9

Laboratory ID	Mass fraction reported [mg/kg]	Mass fraction assumed corrected for recovery	Mass fraction used [mg/kg]	Reported recovery [%]	Reported MU (k=2) [mg/kg]	z-score	zeta-score
27	2.5	NO	2.7	94	0.5	-1.2	-2.0
28	3.8	YES	3.8	90	0.1	1.4	6.3
29	4.2	NO	4.9	85		4.1	
30	3.0	YES	3.0	83	1	-0.4	-0.4
31	1.5	NO	1.6	93		-3.6	
32	4.0	YES	4.0	69		1.9	
33	3.2	YES	3.2	100	0.84	0.1	0.1
35	2.0	NO	3.4	59	1.2	0.5	0.3
36	3.9	NO	4.2	92	2.5	2.5	0.8
37	2.9	NO	3.0	96	0.61	-0.4	-0.5
38	2.5	YES	2.5	79		-1.6	
39	3.2	YES	3.2	102		0.0	
40	2.9	YES	2.9	110	0.13	-0.7	-2.6
41	3.0	YES	3.0	100	0.54	-0.4	-0.6
42	3.0	YES	3.0	103	0.13	-0.4	-1.7
43	1.7	YES	1.7	97	0.17	-3.4	-12.3
44	3.8	NO	3.8	100	0.04	1.4	7.1
45	2.4	NO	3.2	76	0.21	-0.1	-0.2
46	2.9	YES	2.9	95	0.2	-0.7	-2.1
49	4.0	NO	4.0	99.2	1	2.0	1.7
50	4.1	YES	4.1	90	0.62	2.1	2.9
51	3.3	YES	3.3	76	0.2	0.3	0.9
52	2.7	NO	2.7	101	0.9	-1.2	-1.1
53	6.0	NO	6.7	90	2	8.1	3.5
54	4.0	YES	4.0	90	0.8	1.9	2.0
55	4.0	NO	8.0	50	1	11.2	9.5
56	3.0	YES	3.0	99	0.06	-0.4	-2.0
57	3.3	YES	3.3	98	0.5	0.3	0.5
58	2.7	YES	2.7	32	0.11	-1.1	-4.7
59	5.0	NO	5.7	88	0.24	5.8	17.0
60	3.2	YES	3.2	78	0.1	0.0	0.2
61	2.0	NO	4.0	50	1	1.9	1.6
62	2.0	NO	2.7	74	0.64	-1.1	-1.4

Laboratory ID	Mass fraction reported [mg/kg]	Mass fraction assumed corrected for recovery	Mass fraction used [mg/kg]	Reported recovery [%]	Reported MU (k=2) [mg/kg]	z-score	zeta-score
63	2.9	NO	2.8	103		-0.8	
64	2.4	YES	2.4	87	0.1	-1.8	-7.9
65	7.8	NO	8.0	97	3	11.3	3.2
67	4.4	YES	4.4	87	1.06	2.8	2.3
68	3.0	NO	2.9	103	1.32	-0.6	-0.4
69	1.5	NO	5.2	29	0.2	4.6	15.2
70	3.3	YES	3.3	97	0.33	0.3	0.6
72	2.6	NO	3.1	85	0.17	-0.3	-1.0
73	2.7	NO	3.0	90	0.81	-0.4	-0.4
74	2.2	NO	2.5	89	0.4	-1.6	-3.3
75	6.0	YES	6.0	103	1	6.6	5.6
76	2.9	YES	2.9	74	0.87	-0.7	-0.6
78	1.6	NO	2.7	59	0.4	-1.1	-2.2
79	1.5	NO	1.5	98	0.62	-3.8	-5.1
80	3.3	NO	3.3	100	0.65	0.3	0.4
81	2.3	YES	2.3	85	0.91	-2.0	-1.9
82	2.8	NO	3.3	86	0.89	0.2	0.2
84	3.5	NO	3.0	118	0.5	-0.5	-0.8
85	3.0	YES	3.0	78	1.5	-0.4	-0.2
86	3.1	YES	3.1	87	0.19	-0.2	-0.6
87	3.5	YES	3.5	102	0.13	0.7	3.0
88	4.0	YES	4.0	68	1.04	1.9	1.6
89	3.0	NO	3.2	94	0.28	0.0	0.1
90	3.8	YES	3.8	92	0.49	1.4	2.4
91	1.0	YES	1.0	101	0.3	-5.1	-12.6
92	3.1	NO	3.2	97	0.2	0.0	0.1
93	3.0	NO	3.4	88	0.36	0.5	1.2
94	2.7	NO	3.1	88		-0.3	
96	3.5	NO	4.1	85	1.9	2.2	1.0
97	2.6	NO	2.7	95	0.33	-1.0	-2.4
98	2.9	YES	2.9	94	0.47	-0.7	-1.1
99	3.1	YES	3.1	95		-0.2	
100	3.6	NO	3.6	100	0.5	1.0	1.6

Laboratory ID	Mass fraction reported [mg/kg]	Mass fraction assumed corrected for recovery	Mass fraction used [mg/kg]	Reported recovery [%]	Reported MU (k=2) [mg/kg]	z-score	zeta-score
101	3.9	NO	4.0	97	0.94	2.0	1.8
102	3.3	YES	3.3	89	0.1	0.3	1.2
103	2.8	NO	3.6	78	0.3	1.0	2.4
104	3.1	YES	3.1	100	1.03	-0.2	-0.2
105	2.8	YES	2.8		0.66	-0.9	-1.1
106	3.4	YES	3.4	106	0.6	0.5	0.7
107	4.2	YES	4.2		0.4	2.4	4.7
108	3.2	YES	3.2	102	0.22	0.0	0.1
109	3.0	YES	3.0	97	0.12	-0.4	-1.7
110	3.4	NO	3.2	107		0.0	
111	2.7	NO	3.0	90	0.54	-0.4	-0.6
112	3.9	NO	3.9	100	0.04	1.7	8.2
113	3.2	YES	3.2	104	0.65	0.1	0.1
114	4.6	NO	4.4	105	2.5	2.8	1.0
115	1.8	YES	1.8	89	0.28	-3.2	-8.4
116	3.0	YES	3.0	101	0.36	-0.4	-0.9
117	4.6	NO	5.5	83	0.75	5.5	6.1
118	2.4	NO	2.2	109	1.25	-2.3	-1.6
119	3.4	NO	3.4	100	0.2	0.5	1.7
120	3.0	YES	3.0	96	0.51	-0.4	-0.7

12. ANNEX C

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
1	0.5	acetic acid/acetonitrile, 50/50	50	shaking (vortex)	2	no	20	LC		150	3.000	4	phenomenex, synergi RP polar 4µ, 80A	A: water (ammonia acetate)/methanol B: acetonitrile	0-1 min 10%A to 4 min 100% A 4-6 min 100% A 6.5-15 min 10% A	MS	MS/MS	2,4,6-triaminopyrimidine	measurement uncertainty 50% for both samples
2	1	Acetonitrile/ Water (50/50)	25	Geno Grinder	3	Mixed Mode Cation Exchange SPE	10	LC	LC (HILIC)	150	2.100	5	ZIC HILIC (Merck SeQuant)	1. Mobile Phase A: 5% 10mM ammonium acetate : 95% acetonitrile a For example, 50 mL of 10 mM ammonium acetate mixed with 950 mL of acetonitrile 2. Mobile Phase B: 10mM ammonium acetate dissolved in 50:50 acetonitrile:water	a 1 - 10 minutes: 100% A at 0.3 mL/min b 10.1 - 15 minutes: 50% A and 50% B at 0.6 mL/min c 15.1 - 20 minutes: 100% A at 0.6 mL/min d 20.1 - 30 minutes: 100% A at 0.3 mL/min	MS		15NC3 Labeled Melamine	
3	1	Diethyl amine/Acetonitrile/Water 10/50/40	20	Head over Head	30	no	200	GC		30	0.250		Restek RTX-50	Helium	initial temp: 110 C initial time: 2.0 min ramp: 6 deg/min temp: 190 C hold time: 0.0 min ramp: 20 deg/min temp: 290 C hold time 10.0 min	MS		13C3 Melamine; 15N3 Cyanuric acid	- For sample A (milk powder) the volume reported under [7] was 10 µl. With 200 µl the derivatisation yield was poor. - The recovery is the average recovery determined in during analysis of different samples over time, fortified at different concentration levels. The MU was calculated from the RSD(reproducibility) of 23% which results in a relative MU of 46% with k=2. - The method was based on: FDA Laboratory Information Bulletin LIB No. 4423, Volume 24, October 2008 GC-MS Screen for the Presence of Melamine, Ammeline, Ammelide, and Cyanuric Acid - Cyanuric acid, Ammelide and Ammeline were also determined but not detected.

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4	0.5	Aectonitril-H2O (50/50)	20	20min ultrasonic bath , 20min shaking	40	30min deep freezing to separate fat; 100ul are diluted with 900µl water; if necessary membranefiltration	5	Other	1.) HPLC-MS/MS for survey (RT=3.8 min); 2.)quantification on UPLC-MS/MS-System (RT=0.9 min)	100	2.100	2	Waters ACquity BEH C18, 1.7µ	A: 5mM CH3COONH4 in water- B:ACN	100%A (2min) to 100% B within 5 min	MS	MS/MS, 2MRM		Calculation via external calibration; for recovery estimation all samples are spiked at two different levels
5	1	Acetonitrile / Water (50 / 50)	10	shaking, sonication	45	no clean-up	2	LC		100	2.100	2	Acquity UPLC HSS T3	Acetonitrile / Heptafluorobutyric acid (4.26g/L)	0min: 2% ACN / 98% HBFA 1min: 2% ACN / 98% HBFA 4min: 80% ACN / 20% HBFA 5min: 80% ACN / 20% HBFA 5.1min: 2% ACN / 98% HBFA	MS	MS/MS	13C3-, 15N3 - Isotope	
6	0.5	DEA/water/ acetonitrile(10/40/50)	20	ultraturax, sonication, centrifugation	31	filtration using 0.45 um nylon filter	5	GC		30	0.250	0	CP Sil 8CB Low Bleed/ MS	He	85oC (hold 3min) to 320oC at 15oC/min (hold 2min)	MS	MS/MS	benzoguanamine	Particle size for column[µm]: 0.25
7	0.5	0.2 M Perchloric acid	10	Shaking	30	Waters Oasis MCX 3 cc (60 mg) tubes, conditioned with 3 ml 5% NH4OH in MeOH, 3 ml 1% HCl in MeOH, 3 ml MeOH, 3 ml UPW, load 3 ml sample, wash 3 ml UPW, wash 3 ml MeOH, elute 3 ml 5% NH4OH in MeOH, evaporate to dryness,	1000	LC		100	2.100	2	Waters Acquity UPLC C18 BEH HILIC	90:10 ACN:UPW with 10 mM ammonium acetate added	Isocratic, flow rate 0.3 ml/min, column T=35 degC	MS		None	The measurement uncertainty has not been calculated for these matrices.

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						reconstitute in 1 ml UPLC mobile phase													
8	1	Trichoacetic acid 2% : acetonitrile (1:9)	5	shaking	15	SPE cartridge	10	LC		250	4.600	5	Discovery C18	33 mM octanesulfonic acid in phosphate buffer:acetonitrile 85:15	Gradient, phase B - acetonitrile 100%	UV			The results are not correct for recovery
9	1	acetonitril / water / trichloroacetic acid (90 / 9.8 / 0.2)	10	shaking	0	Yes, SEP Pack	10	LC		250	4.600	5	Zorbax	Water / acetonitril	isocratic	UV		No	No
10	0.1	Acetonitrile / water (70:30)	50	automated shaker	10	Centrifugation followed by a direct injection the resulting supernatant	50000	LC		150	2.100	5	Merck - SeQuant ZIC-HILIC	A: ammonium acetate 10 mM in water B: acetonitrile	0-7 min: 5%A; 7-10 min: 80%A;10-11 min:80%A;11-11.5 min: 5%A; 11.5-26.5min 5%A.	MS	MS/MS	yes: [13C3, 15N3] - melamine	- LC parameters given are used for the simultaneous determination of melamine and cyanuric acid. - test portion size is 0.1 g for sample A and 0.5 g for sample B. - expanded uncertainties are given in % - results have not been corrected with recovery
11	0	Methanol / Water = 1 / 1, pH = 4	5	extraction for 15 min, shaking for 5 min, sonication for 15 min	35	centrifugation of extract, 2.5 ml are cleaned up by eluting through a Chromabond HR-X (Macherey-Nagel, 200mg/6ml) SPE-Tube with subsequent dilution to 10 ml	10000	LC		100	2.100	5	Hypercarb II (ThermoFisher Analytic)	Eluent A: Water + 0.005% formic acid Eluent B: methanol 100%	min Eluent A Eluent B Flow 0 90% 10% 300 µl 5 15% 85% 300 µl 7 15% 85% 300 µl 7.1 90% 10% 300 µl 16 90% 10% 300 µl	MS		15N3-melamine	Our quantification is based on multiple standard-addition and work-up. For that it is necessary to perform a screening in order to calculate, which concentrations have to be added. The LC-MS/MS-instrument was out of order during most of January so that we could only calculate the amounts for the standard-addition following ELISA, which obviously delivered low values. Therefore we only have one result for calculating the backing-mix and the measurement uncertainty is estimated only. Recoveries are calculated by comparison of the signal of solvent standards (100%) and the signal difference between spiked and unspiked samples (x%).

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12	0.5	water+acetonitrile+DEA (4+5+1)	10	VORTEX + SONICATION	31		15	LC		100	3.0000000	3	ATLANTIS HILIC SILICA	AMMONIUM FORMIATE pH3 /ACETONITRILE	ISOCRATIC WITH FLOW GRADIENT FROM	MS		MM 13C3, 15N3	
13	2	2.5% Formic acid in Water; diluition 1:20 by ACN	14	shaking and sonication	30	centrifugation and filtration, proteins precipitation by Acetonitile diluition	25	LC		150	2.100	3	Phenomenex Hilic	ACN/10 mM Ammonium Acetate buffer	90/10	MS		Melamine 13C3 15N3	
14	1	24 ml Acetonitrile/water (50/50) + 1 ml 1 N HCl	25	shaking + vortex	2	yes, refrigeration + SPE	10	LC		100	2.100	2	Acquity UPLC	Acetonitrile:water (95:5)+10 mM NH4Ac	isocratic	MS		yes, 13C3H615N314N3	
15	1	50/50 ACN/Water 3% formic acid	100	shaking sonication	30	no	10	LC		150	2.100	2	Acquity UPLC BEH HILIC 1.7um	ACN/H2O		MS			
16	0.5	Diethylamine/Water/Acetonitril (10/40/50)	20	Ultrasonics	30	Centrifugation, Filtration	500	GC		30	0.250		Zebron ZB-5ms	He	75°C for 1min; 15°C/min; 300°C	MS		Melamin (C13, N15)	The recovery was not applied to result!
17	1	Water : 1N HCl (24:1)	25	Rotary mixing followed by centrifugation.	20	Liquid-liquid extraction followed by mixed mode reverse phase cation exchange solid phase extraction.	1000	LC		100	2.100	2	Waters Acquity UPLC BEH HILIC	Mobile phase A: 0.5 mM ammonium formate and 0.01 % formic acid in water. Mobile phase B: acetonitrile	Time (min) Flow Rate (mL/min) %A %B Initial 0.17 9 91 2 0.17 9 91 4 0.17 40 60 10 0.17 40 60 10.5 0.25 9 91 12.5 0.25 9 91 12.6 0.17 9 91 15	MS	positive ESI MS/MS in multiple reaction monitoring mode	Recovery standard : 13C3-Melamine Performance standard : 15N3,13C3-Melamine	The estimation of apparent recovery was calculated by the use of a recovery standard (13C3-Melamine) added to the samples. The estimation of Measurement Uncertainty is 10.5 ± 2.0mg/kg for the milk powder and 3.2 ± 0.6 mg/kg for the backing mix.

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															0.17 9 91				
18	1	Acetonitrile/ water (50/50)	100	mix (1 min), sonication	30	yes, according to Romer Labs Application Brief (Rapid Accurate Quantitation of Melamine... using Mycosep 224 Clean-up)	10	LC		150	4.600	5	Macher ey-Nagel	A: water (0.1% NH4OAc), B: Acetonitrile	20% B to 8 min. - 95% B from 10 to 15 min. - 20% B from 16 to 20 min.	MS		No	Expected RT: 3.8 min MS/MS (MRM) detection (127 --> 68; 127 --> 85)
19	2	2.5 % Formic acid Acetonitrile	20	sonication, shaking	30	no	5	LC		100	2.100	4	Waters X-Bridge HILIC	A: 10 mmol Ammonium acetate/Acetonitrile 5/95 B: 10 mmol Ammonium acetate/Acetonitrile 50/50	Gradient: 0 - 0.5 min %A = 100, 0.5 - 3.5 linear to %B = 50 hold 3 min	MS		yes Melamine 13C3 (Witega, Berlin)	
20	0.5	2.5 % formic acid	10	shaking, dilution (20x)	30	no	5	LC		150	2.100	5	ZIC - HILIC	A - 0.1% formic acid, B - acetonitrile (0,1% formic acid)	0 min - A:B 5:95, 4 min A:B 40:60, 7 min - A:B 40:60, 7.1 min A:B 5:95,	MS		no	method of standard addition was used, recovery was not determinated
21	0.5	Acetonitril/w ater (50/50)	20	shaking, sonication	30	MycoSep 226 (RomerLab s), dilution, membrane filtration	10	LC		100	2.000	3	Pheno menex Luna Hilic	A: MeCN; B:10mM NH4-Acetate	0-2min: 95%A; 2-3min 50%A; 3-7min50%A; 7-8min 5%A; 8-10min 5%A; 10-11min 95%A; 11-20 min95%A	MS	LC/MSMS	Melamin 13C3, 15N3	
22	0.3	ACN/H2O 50:50	5	Ultraturrax	1	SPE Strata XC 60 mg, 3 ml Phenomen ex	1000	LC		150	2.000	3	C18 Pheno menex	A: 25 mMol Ammonium acetate, 25 mMol Acetic acid in H2O B: Acetonitrile C: Methanol	min - %A - %B - %C 0 - 65 - 30 - 5 5 - 30 - 65 - 5 7 - 5 - 90 - 5 12 - 80 - 15 - 5	MS		Melamine 13C3 labelled	

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23	1	HCl 0.1mol/l	10	shaking + sonication	10	SPE on strong cation exchange column	2000	LC		100	2.000	3	Phenomenex Luna HILIC	CH3CN/ 0.1 mol/l Ammonium formate at pH=3.2 90/10 v/v	isocratic	MS		13C3 15N3 14N3 H6	
24	0.5	water/acetonitrile/trichloro-acetic-acid (w=5% in Water) (10 / 10 / 80)	50	sonication	30	-20C for 16h centrifugation 14000 rpm for 10 min at 4°C	10	LC		100	2.1000000	5	SeQuant Zic-pHILIC	A: 10mM ammoniumacetate, pH 7.2 B: acetonitrile	0min 97% B; 10min 80% B; 10,5 min 45% B; 20,5min 45% B; 20,6 min 97% B; 31min 97% B	MS	MS/MS	Melamine (13C3/amino15N3)	From step 6 to 7 dilution with acetonitrile factor 10 or 50
25	0.5	Diethylamine/water/acetonitrile 10/40/50	20	Shaking	30	No	0	GC		30	0.250	0	Varian FactorFour VF-5-ms-30	Helium 5.0	100 C hold 1 min. to 210 C 10 c/min to 300 c 30 c/min hold 5 min.	MS		2,6-Diamino-4-chloropyrimidine	
26	2	Acetonitrile/ Water (50/50)	25	Sonication	60	SPE - OASIS MCX 3cc/60mg	1000	LC	UPLC system	150	2.100	2	WATERS ACQUITY BEH HILIC - 150x2.1 mm - 1.7 µm (UPLC)	10 mM Ammonium Acetate in Acetonitrile/Water (95/5)	Isocratic	MS	MS/MS - MRM transitions	Melamine (13C3, Amino 15N3)	VALIDATION PARAMETERS on MILK POWDER LOD : 0.01 mg/kg LOQ : 0.1 mg/kg Recovery: 97% CV repeatability : 4% CV reproductibility : 6% Uncertainty : 12 % RECOVERY on DIFFERENTS MATRIX (cereals, blood meal, eggs...) : 88 % - 110 %
27	0.5	Water/Trichloroacetic acid/Acetonitrile (50/1/49)	10	Shaking and centrifugation	1	Yes, we use MCX SPE cartridges.	20	LC		250	4.600	5	Discovery C18 (Supelco)	Octanesulfonic acid in phosphate buffer/Acetonitrile (85/15)	Increasing acetonitrile concentration in mobile phase (up to 40%) for column washing	UV	Diode Array Detector		
28	0.3	Water/Acetonitrile (50/50)	40	Sonication	30	Carrez I + II	20	LC		125	4.000	5	LiChrospher	Water (0,1 % FA)/ACN (99,5/0,5)	Isocratic	MS			Quantification by external matrix calibration

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29	0.1	0.2 M trichloroacetic acid aqueous	10	Shake	30	Yes, Oasis MCX SPE (150mg, 6ml) 2.0 ml extract applied, elute with diethylamine in acetonitrile	400	LC		150	2.100	5	SIELC Obelisc M	A: 20mM ammonium acetate with 0.1% acetic acid (aqueous) B: acetonitrile	Time 0min: 5% A, 95% B Time 5min: 50% A, 50% B Time 9min: 50% A, 50% B Flow rate: 0.25 ml/min	MS			
30	2	acetonitril/water (50/50)	20	shaking 150 rpm	30	1 ml of the supernatant of the extraction described under 2-5 is diluted with 10 ml 4% formic acid in acetonitril, shaken again 15 min, 200 rpm, centrifuged and the supernatant is used for LC-MS/MS determination.	5	LC		100	2.100	3	Waters Atlantis HILIC Silica	Mobile phase A (95% ACN / 1 mM Ammonium acetate Mobile phase B (50% ACN / 10 mM Ammonium acetate	0 min 0 % B flow 0,2 ml/min 10 min 100 % B flow 0,2 ml/min 15 min 100 % B flow 0,2 ml/min 16 min 0 % B flow 0,2 ml/min 25 min 0 % B flow 0,2 ml/min	MS	LC-MS/MS in MRM mode	isotope labelled melamine 13C3, 15N3	Measurement uncertainty is 35% and includes a bias contribution and a reproducibility contribution. MU was calculated as described in the Dutch standard NEN7779 which is proposed to CEN to be considered as an European standard. The figures listed above are calculated by taking this percentage from the amount determined in the sample
31	0.5	acetonitrile: water 1:1	10	shaking	30	centrifugation for 10 minutes at 4500 rpm	10	LC		250	4.600	5	Zorbax SAX	methanol (5%) :phosphate buffer 20 mM, pH 3.7 (95%)	gradient: 5:95 changing to 15:85 in 7 minutes, 7-15 minutes returning to original 5:95 ratio	UV		none	measurement uncertainty is not estimated since the method is not accredited; sample mass (~ 5g) is too small; our usual sample mass is ~ 2 g for a sample

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32	0.5	0,1% TFA in water	10	sonication and shaking	10	filter, SPE (Strata-X-C 60mg)2and 5 ml, respectivel y	1000	LC		100	0.000	3	Pheno menex, Luna Hilic 200 A	A: 25 mM acetic acid, 25 mM ammoinium acetate in water B: acetonitril V/V: 30/70	isocratic, 0.2 ml/min 20 °C	MS		Melamin, 13 C3 99% Amino 15 N3 98%	
33	1	Water	50	Shaking	60	SPE (Strata XC)	10000	LC		150	4.600	3	Atlantis-Hilic Silica	A: Water B: Acetonitrile C: 1 mM NH4OC pH 5, 0.5 ml/min, T = 40 C	Time - A - B - C 0 - 5% - 90% - 5% 10 - 85% - 10% - 5% 11 - 5% - 90% - 5% 20 - 5% - 90% - 5%	MS	MS/MS	13C3 Melamine	
35	0.5	10 % diethylamin e + 40% water + 50% acetonitrile	20	Sonication	30	No	500	GC		30	0.25 mm	1.0 u film thickness	Agilent DB-5 MS	Helium	100-200 deg C at 4 deg C/min (1 min intitial hold), 200 - 320 deg C at 15 deg C/min. Total run time 24.83 mins.	MS		2,6-Diamino-4-chloropyrim idine used as internal standard taken through extraction method.	The method used is for quantitation at 10 mg/kg and above. The uncertainty calculated reflects the trace level reporting range between 2.5 mg/kg and 10 mg/kg and the high value reflects this. The low recovery for the baking mix is due to baking POWDER being used as a positive control instead of a mix. It is believed the salt content of the powder interfered with the final recovery.

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36	0.5	10:40:50 Diethylamine : water : acetonitrile	10	agitation & sonication	34	0.45um nylon filter, TMS derivatization	200	GC	200uL of extract dried and derivatized to a 0.5mL total volume; 1uL of derivatized soln injected	30	0.250	25	Agilent HP5-MS	He	100 C, hold 1 min, ramp at 10 C/min to 210 C, ramp at 30 C/min to 320 C, hold 10min	MS		4-chloro-2,6-diaminopyrimidine	method ref: US FDA Library Information Bulletin #4423, GCMS Screen for the Presence of Melamine, Ammeline, Ammelide and Cyanuric Acid. The samples were extracted once and analyzed on two GCMSDs. The reported result is an average of the two sets of data (individual data sets attached as separate spreadsheet). The measurement uncertainty is calculated from accumulated data from 2 ug/g fortification recoveries between Dec 2008 and Jan 2009. The fortifications were performed with a broad range of products, including milk powder/liquids.
37	0.5	50/40/10 (acetonitrile/water/diethylamine)	20	vortex 1 minute + sonication 30minutes	31	NO	1	GC		30	0.250	0	Agilent HP-5MS (30m x 0.25mm x 0.25um)	Helium	100C (hold 1 min) to 210C @ 10C/min then 210 to 300C @ 30C/min (hold 10 min)	MS		Yes, 4-Chloro-2,6-diaminopyrimidine	The uncertainty estimate is listed in percent.
38	1	Acetonitrile/ Water/Diethylamine (50/40/10)	25	blending	1	No	250	LC		150	4.600	4	Phenomenex Synergi RP-Polar	A = 10 mM ammonium acetate B = Methanol C = 10 mM ammonium acetate in 0.1% formic acid	Gradient elution, starting at 80/15/5 A/B/C, held for one minute, then ramped to 30/65/5 at 5 minutes, held until 5.1 minutes, then ramped to 5/90/5, held until 7 minutes, then returned to initial conditions and re-equilibrated for 5 minutes.	MS		15N3-Melamine	Samples were analyzed using slight modifications of the method published in J. Agric. Food Chem; 56(17) 7593. Some problems with filtration of the final extract were encountered. Samples were analyzed twice with nearly identical results.
39	0.1	Acetonitrile	10	Shaking	1	SPE (SCX)	500	LC		250	2.000	5	TOSOH Bioscience	A: Ammonium Acetate 10 mM B: Acetonitrile	Time [min] - Flow [ul/min] - A% - B % 0 - 350 - 30 - 70 0.1 - 350 - 30 - 70 5 - 250 - 80 - 20 7 - 250 - 80 - 20 7.5 - 250 - 30 - 70 8.5 - 350 - 30 - 70 13 - 350 - 30 - 70	MS		13C3 15N3 labelled Melamine	Test portion: 0.1 g milk powder, 0.5 g baking mix

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40	0.5	Acetonitrile/ water 50/50	10	sonication + vortexing	10	no	1	LC		100	2.100	3	Waters Atlantis HILIC	Acetontrile/Water 95/5 + 10 mmol/l ammonium acetate	isocratic + isothermal	MS		Melamine-15N3	
41	0.1	Acetonitrile/ Water	4	shaking and sonication	30	no sample clean-up	50	GC		30	0.250 0000 0	0	Zebron ZB-5MS	Helium	120°C (1min) ; 10°C/min until 320°C (2 min)	MS	(MS/MS)	Melamine 13C3 15N3 ; Acid cyanuric 13C3 15N3	Use of benzoguanamine as external standard
42	0.5	Acetonitrile/ water (60/40)	25	Shaking	10	Oasis MCX cartridges were used to clean up the extract.	5	LC		10	2.100	2	Waters	Acetonitrile: 20 mM Ammonium Acetate (90: 10, v/v)	Isocratic	MS		13C3-15N3-Melamine	
43	0.3	Acetonitrile/ Water (50/50) (pH 3 Acetic acid)	25	90 min sonication 30 min shaking 1 min blending (with "UlraTurax")	121	SPE clean-up with "Strata X-C" Eluate with Diethylamin /Acetonitrile /Water dry under N2, solve with HPLC eluent	10	LC		150	2.100	5	Agilent Zorbax RX-SIL	A: Water with 5 mmol/L NH4COOH (pH 3 Acetic acid) B: Acetonitrile	Start: 0 % A; 100 % B End: 5% A; 95 % B Column cleaning: 50 % A; 50 % B	MS	MS-MS	13C3,15N3-Melamine	

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44	0.2	water/acetonitrile/1.0M HCl (12:12:1)	1	vortex	20	Solid phase extraction with Waters MCX 1c.c cartridge Condition: 1 mL Methanol and 1 mL DI water Loading : supernatant Wash: 1 mL 2.5 % formic acid and 1 mL acetonitrile Elute : 5 % ammonia in acetonitrile	15	LC		250	4.600	5	Luna C18 ((Phenomenex, Torrance, CA, USA,)	0.1M SDS in water (adjusted to pH=3.0 with 1.0 M phosphoric acid) / Acetonitrile	39% Acetonitrile / isocratic	UV			
45	0.5	Acetonitril / Water (50/50)	50	treating with UltraTurrax for 1 min	15	SPE with Phenomenex StrataX-C: Aliquot of extract is acidified with 1 M HCl to pH 1.9, transfer to SPE column, washing with 0.1 M HCl, washing with Methanol, elution with 10% NH3 in methanol	1000	LC	injected volume 15 µL	150	2.100		SeQuant ZIC-HILIC, 200 Angstrom	A: 10 mM Ammoniumacetat in Water B: 10 mM Ammoniumacetat in Acetonitril	0.0 min 97% B 0.1 min 97% B 5.0 min 20%B 5.5 min 3% B 5.6 min 97% B 10.0 min 97% B	MS	MS/MS		3 diagnostic fragmentations: 127.0/85.0 amu intensity 100% 127.0/68.0 amu intensity 30% 127.0/60.0 amu intensity 5%
46	0.3	acetonitrile/ water (50/50; v/v)	10	agitation	10	no	0	GC		30	0.250		J&W	Helium	80°C, 30°C/min ad 320°C, 320°C for 10 minutes	MS		Melamin-D3	

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
47	1	Aqueous solution of NaH2PO4, 5 mM, pH=5,0	75	Sonication	20	No	20	LC		250	4.600	5	Luna CN, Phenomenex	Isocratic 5 mM NaH2PO4 (aq), pH=5.00	isocratic/isothermal(30 ºC)	UV		Resorcine	Dear coordinator, we can not provide data for the backing mix due to a malfunction of the instrument. The uncertainty for the milk powder sample will be provided in the forthcoming weeks.
49	1	2.5% Formic Acid in water	14	15-30 sec (vortex as needed), then sonicate in ultrasonic bath and mix on multi vortex mixer for 30 min each.	120	no	1000	LC		150	2.100	5	Nest Group, Sequant ZIC-HILIC	Mobile Phase A: ACN /0.1% Formic Acid in water (95/5). Mobile Phase B: Ammonium Formate 20mM/ACN (50/50).	Table I. Mobile Phase Gradient Time (min) % A % B Flow rate (mL/min) 0 100 0 0.4 initial 4.2 100 0 0.4 Linear 8.0 65 35 0.4 Linear 8.5 65 35 0.4 Linear 9.0 25 75 0.4 Linear 11 25 75 0.4 Linear 11.2 100 0 0.6 Linear 13 100 0 0.6 Linear 14 100 0 0.4 Linear	MS		no	Question 7: The method employed by our laboratory did not involve a cleanup or concentration step. The initial volume used for the extraction, 14 mL, was diluted before analysis on the LC/MS., i.e. 50 µl + 950 µl (Acetonitrile) -----> 1000 µl (Total Volume)

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
50	2	2.5% formic acid	20	Heating (+/- 70 C) sonicate 5 min. shaking 10 min.	17	no	1000	LC		150	2.100	5	Alltech Hilic	A: 10 mmol/l ammonium formate B: Acetonitril	0 min : 2% A 98% B 5 min : 80%A 20% B 5.5 min 98%A 2% B 5.6 -16 min 2% A 98% B	MS		no	Sample is measured with Standard addition to eliminate matrix effects. Working standards prepared on blanc milk powder to eliminate matrix effects. Sample extracted with 20 ml 2.5% formic acid extract diluted 0.1 ml with 0.9 ml 50% acetonitril Injection volume 5 ul. Extraction with formic acid is required to dissolve the possible melamine/cyanuricacid complex in the sample.
51	1	Acetonitril/ water (50/50)	24	shaking	20	yes, SPE with OASIS MCX	1000	LC		150	2.000	5	Pheno menex	Acetonitril/ water	isocratic	MS		yes, 15N3 Melamine	
52	0.5	Diéthylamin e / water / acetonitrile (10/40/50)	20	shaking (15 min) + sonication (15 min)	30	Yes : centrifugati on + filtration 0.2 µm	200	GC		30	0.250	0	J&W DB-5MS	helium	80°C (2min) - >10°C/min -> 250°C (2min)	MS		2,6-diamino-4-chloropyrim idine	For each material : the result is the mean value of 2 results. The recovery is determinated by standard addition (also 2 preparations added with the equivalent of 10 mg/kg)
53	0.3	10/40/50 DIETHYLA MINE/WAT ER/ACETO NITRILE	10	shaking+so nication	60	Only centrifugati on and filtration of supernatan t using 0,45 um nylon filter	250	GC		30	0.250		HP-5MS	He 6.0	Inlet: 275 C GC: 100 hold 1 min-30C/min-150C hold 1 min-10C/min-300C hold 2 min	MS		2,6-DIAMINO-4-CHLOROP YRIMIDINE	
54	0.5	Water / Acetonitrile (10:90)	10	Shaking	10	No	1000	Other	UPLC	10	2.100	2	BEH HILIC (with particle size of exactly 1.7 µm)	Solvent A = ammonium acetate (20 mM) Solvent B = acetonitrile	Gradient (A% / B%) : at 0 min : 0/100 at 3 min : 30/70 at 3.1 min : 0/100	MS	More precisely, MS/MS	No	Analyses were performed with standard addition method.
55	0.5	Diethylamin e/water/acé tonitrile (10-40-50)	20	sonication	30	Filtration 0,45µm	200	GC	Sylilatio n MSTFA	30	0.250	0	VF 5 MS	Hélium	75° - 320° (15°/mn)	MS		benzoguani dine	

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
56	1	Acetonitrile/ Water (50/50)	20	Shaking	20	SPE	3	LC		100	2.100	2	Acquity (Waters)	Mobile phase A: 10 mM amminiumacetate Mobile phase B: 10mM amminiumacetate i 95:5 acetonitrile / H2O	Time % A % B 0,0 min 0 100 0,8 min 0 100 2,3 min 22 78 2,8 min 22 78 2,9 min 0 100 6,0 min 0 100	MS		13C3, Amino-15N3	
57	0.5	acetonitrile/ water/diethy lamine (5/4/1)	10	sonication	30	no	250	GC		30	0.250	0	Varian VF-5ms	He	75 □(1min)-- 10□/min-- 290□(10min)	MS	MS/MS	yes, 13C-15N-melamine	
58	5	Acetonitrile/ water (50/50)	15	shaking 250 rpm	10	Oasis MCX , 150 mg , 6 mL (P/N 186000255 , Waters)	4000	LC		100	2.100	2	ACQUI TY UPLC BEH HILIC column	5% 100 mM ammonium acetate : 95% acetonitrile	isocratic	MS	LC/MS/M S	13C3,15N3 -Melamine	
59	0.9	PBS Buffer with 10% Methanol	6	Vortex Shaking	2	No	100	None	1 ml of total volume of extracti on(6ml) was diluted to 5ml and 100 micro litre was used for analysis					N/A	N/A	Immuno metric (f.i. ELISA, etc.)	None		
60	0.5	Diethylamin e/Water/Ac etonitril (10/40/50)	20	Horizontal shaking, sonication	30	Precipitatio n with cold Acetonitril, filtration	300	GC	LC-MS-MS method e was also used with the same results	30	0.250	0	DB-5	Helium : GC-MS Ammonium Acetate/ Acetonitril: LC-MS-MS	Standard Method of FDA: GC-MS Agilent QQQ-Method: LC-MS-MS	MS	Also LC-MS-MS method	Melamin N15(3)	

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
61	1	acetonitrile / eau (50/50)	20	shaking 30 s, blending 1 min, shaking 20min, centrigation 4000tr/min 5 min	26	SPE OASIS MCX 6mL,150mg elution Acetonitrile - diethylamine 2%	20	LC		150	2.100	5	sequant ZIC HILIC	a : water- ammonium formiate 20mM b : acetonitrile	gradient : 0 min 95% b, 5 min 50%, 10 min 50%, 12 min 95%, 17 min 95% colon : 24 °C flow : 0.3 mL/min	MS			
62	2	Methanol/Water (50/50)	20	Shaking and sonication	12	SPE strong cation exchange	10	LC		150	3.000	5	Supelco	Mob A: Methanol 95%, Mob B: Water 5%	Isocratic	MS		Yes, cyramozine	
63	1	acetonitrile/ water 50/50% V/V	24	shaking + centrifugation	0	Waters OASIS MCX cartridges (cation exchanger)	1000	LC		50	2.100	2	Waters Acquity BEH HILIC	A: ammonium acetate 10 mM B: ammonium acetate 10 mM in 95/5 acetonitrile/water	0-1.6 min: 100%B 1.6-4.6 min: from 100%B to 78%B (curve 6) 4.6-5.6 min: 78%B 5.6-5.8 min: from 78%B to 100%B (curve 6) 5.8-8 min: 100%B constant flow of 0.3 ml/min during the whole analysis	MS	(MS/MS)	no	samples were identified as A and B, without reference to the matrix: we assume A was the milk powder and B the baked product
64	1	0.01% TFA in Acetonitrile/ Water 50:50	50	Stirring	40	Oasis MCX SPE 1ml/60mg Condition 1)methanol 2)0.1% TFA;Load 1ml extract;Wash 1) 0.1%TFA 2) Methanol;Dry;elute 1ml 0.1% ammonia in methanol	5	LC		150	2.0000000	3	Phenomenex HILIC	Acetonitrile/20mM Ammonium Formate	95% Acetonitrile to 85% Acetonitrile in 10 minutes	MS	MS-MS	No	This was our first try of the method and used spike/single point standard addition for quantitation. Method uncertainty looks to be high and suggests an internal standard approach would be better.

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
65	0.5	acn/H2O (70:30)	50	vortex+sonication	30	no	10	LC		100	2.100	4	Waters Atlantis HILIC	ammonium formiate buffer:ACN	isocratic	MS			
66	1	water	0	shaking	0	centrifuge and dilute x5	150	None			0.000		0	0	0	Immuno metric (f.i. ELISA, etc.)	0	0	We believe there are interfering substances in the backing material so that recovery of melamine was very low.
67	0.5	Acetonitrile 50 : water 40 : diethylamine 10	20	Sonication	30	No	500	GC		30	0.250	0	J + W HP5-Msi	Helium	120°C/1 min, 30°C/min to 180°C 2min, 5°C/min to 215°C 1.5 min, 30°C/min to 320°C 1 min.	MS		Yes - benzoguanamine	2µL injection split 10:1. 250°C injection. Ions monitored : IS 316, 331, 333. Melamine : 327, 342, 344. Results ARE CORRECTED for method recovery. Final vol made up of 200 pyridine, 200 BSTFA/TCMS, 100 IS in pyridine
68	0.5	Water/acetonitrile/diethylamine (40/50/10)	20	ultrasonic extraction 15min followed by shaking 15min	30	no	200	GC		30	0.250		Grace	helium	75°C (1min hold) 15°C/min to 310°C (5min)	MS		benzamide	
69	0.5	Acidified water (HCL) : Acetonitrile 1:1	30	Shaking followed by Sonocation	30	No	100	LC		150	2.100	3	Atlantis HILIC	A: Water + 10 mM Ammonium Acetate B: Acetonitrile + 10 mM Ammonium Acetate	0.01 min 75 % B 3.5 min 75 % B 6.5 min 50 % B 8.5 min 50 % B	MS		API 5000 LC-MS-MS	
70	1	Acetonitrile: Water:Diethylamine(3:1:1)	20	shake 30 minutes and sonicate for 1 hour	120	sample then further dilute 4 time in acetonitrile	5	LC		150	2.100	3	Waters HILIC column 150X2.1 3µm	A: 20 mM ammonia acetate B: 1% Formic acid in Acetonitrile:Isopropanol 9:1	40 degree flow 0.3ml/min gradient from 0-7.5 minutes, Mobile phase B drop from 93% to 50% and 8.5 minutes back to 93%	MS		13C3H615N3N3	

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72	1	Acetonitrile/ Water 1+1	50	Ultrasonic, 70 C	30	Addition of TCAA, -18 C over night, Dilution	4	LC		150	2.100	5	Zic Hilic	A: Acetonitrile B: Ammonium acetate 10 mmol pH 7.2	0 - 10 : 7% B - 20% B 10 - 10.5 : 20% B - 55% B Reequilibration	MS	MS/MS		
73	0.5	Diethylamine/Water/Acetonitrile (10/40/50)	20	sonication	30	---	1	GC		30	0.000	0	HP-5ms	He	105 °C(1min)-->10 °/min-->320 °C(5min)	MS		Benzoguanamin	
74	1	5% TCA in water	5	shaking 10 min	10	SPE using 1cc Strata X-C from Phenomenex	1000	LC		100	3.000	3	Waters Atlantis HILIC Silica	ACN:100mM Ammonium Formate pH 3.2 (9:1)	isocratic mode	MS		No	Sample was not homogenius!
75	0.1	DEA : H2O : Acetonitrol (10:40:50)	20	sonication	30		200	None		30	0.250	0	Agilent	Helium	temperature programme	MS		melamin deuteriert	
76	1	2% NH4OH	10	sonication	30	no	5	LC		100	2.100	4	Xbridge HILIC	90/10 ACN/H2O 10 mM NH4OAC	isocratic	MS		melamine N15	
78	0.5	acetonitrile/ water 50/50	20	sonication	30	SPE-column, Waters Oasis MCX	100	GC		30	0.250		DB-5MS	Helium	100 ºCx1 minx60 ºC/minx180 ºCx1 minx10 ºC/minx300ºCx5 min	MS			
79	1	Methanol / H2O 60:40	5	Shaking (Vortex), Ultrasonic	1	Filtration 0.45 µm	150	None						None	None	Immuno metric (f.i. ELISA, etc.)			ROMER LAB AgraQuant Melamine Assay Order#: COKAQ9300
80	1	ACN/H20 (50/50)	10	shaking	10	LLE with CH2Cl2	1	LC		150	3.000	5	HILIC Phenomenex			MS			

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81	0.5	diethylamine/water/acetonitrile (10/40/50)	20	shaking, sonication and centrifugation	40	filtration - nylon filter 0.45 µm	500	GC		30	0.250	0	Varian, Factor Four VF 5 ms	helium	Initial temperature: 80 °C for 2 min Rate 10 °C/min Final temperature: 250 °C for 2 min	MS		2,6 - diamino - 4 - chloropyrimidine	Calibration curves were done in matrices.
82	0.1	Acetonitrile/ Water (5/1) + 10mM Ammonium acetate + 0.005% Aceticacid	10	shaking	60	no	5	LC		50	2.000	3	Luna HILIC	A: Water + 10mM Ammoniumacetate + 0.005% Aceticacid B: Acetonitrile + 10mM Ammoniumacetate + 0.005% Aceticacid	3min 100%B / 7min 20%B / 7.5min 0%B / 10.5min 0%B / 11min 100%B / 15min 100%B	MS	MS/MS MRM-Mode	Glufosinate	
84	1	Acetonitrile/ water (50/50)	15	Sonicate & vortex	30	No	500	LC		100	2.000	3	Waters Spherisorb S3 Amino	Acetonitrile/Ammonium acetate	Gradient	MS	Tandem MS detection	Yes	Results presented here are NOT CORRECTED FOR APPARENT RECOVERY
85	5	Acetonitrile/ water (50/50)	40	Shaking	60	No	1000	LC		150	2.100	3	HILIC	Ammonium acetate/Acetonitrile	Normal phase gradient	MS		Yes, 13C15N-melamine	
86	1	Water : Acetonitrile : 1M HCl = 12 : 12 : 1	25	shaking	10	SPE column, Bond Elut Plexa PCX (varian), Loaded sup. volume is 3mL. Cleaned, dried sample is dissolved with 3mL of LC mobile phase.	3000	LC		100	2.000	3	varian, Polaris NH2	80% Acetonitrile and 20% H2O (0.01% formic acid, 10mM ammonium acetate)	isocratic, isothermal (40oC)	MS		13C3-15N3-melamine	

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87	1	Acetonitrile/ Water (50/50)	20	shaking	60	SPE, medium cation exchange, FDA-Method LIB.No. 4422	10	LC		100	2.100	3	Waters Atlantis HILIC	Acetonitrile / 10mmol Ammoniumacetate (95/5)	none	MS	MS/MS	15N3-Melamine	
88	0.5	Perchloric Acid 0.2M	10	sonication and centrifugation	15	SPE Oasis MCX	2000	LC		100	2.000	3	Phenomenex Hylic	Ammonium Formiate 20 mM Acetonitril	from 5% A to 50% A in 10 min flow 0.2	MS			
89	0.5	Acetonitril/water/1nHCl (48/48/4)	25	sonication	30	MCX-SPE	1000	LC		50	2.000	3	Atlantis HILIC	A: Acetonitrile 0.1% acetic acid B: Acetonitrile/20mmol ammonium acetate (1:1)	100%A in 5 min to 50%A	MS			
90	0.2	ACN/Water (50/50)	10	vortexing and sonication	30	SCX	5	LC		150	2.100	5	ZIC-HILIC	Phase A: ACN - Ammonium formate 20mM (95:5) Phase B: Ammonium formate 20mM	98% A for 5 min, then to 60% A in 10 min, linear gradient. Flow 0.15 mL/min	MS			Cyanuric acid not detected (< 250 ng/g)
91	1	Acetonitryl/ water (50/50)	10	vortex, sonication	15	no	5	LC		150	3.000	5	Hilic	A: acetonitryle+ 10mM amonium acetate B: water + 10mM amonium acetate	gradient: 0 97 (A) 5 20 (A) 8 97 (A) 15 97 (A) 40 isothermal	MS		melamine-3N15	
92	0.5	Acetonitrile and water (50/50)	10	Vortex for 10 second and sonication for 20 minutes	21	Fat removal with dichloromethane	5000	LC		100	2.100	2	Acquity Hilic	10 mM ammonium acetate in 90/10 acetonitrile/water	isocratic	MS		no	

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
93	1	0.1% trifluoroacetic acid in water at 50 degrees C.	50	Shaking and blending	10	Yes, C18 and ion exchange cartridge.	20	LC		150	4.600	3	Phenomenex HILIC	90% Acetonitrile 10% 100mM Ammonium Formate pH 3.2	isocratic at ambient	UV		No	The uncertainty given is in mg/kg for each result.
94	1	20 mM Phosphate buffer (pH 7.2) : MeOH 9 : 1	100	Sonication	2		100	Other	ELISA (Abraxis Melamine Plate Kit)					/	/	Immunometric (f.i. ELISA, etc.)			
96	1	DEA+H2O+ACN (10/40/50)	5	Sonoication	0	NO	0	None		30	0.250	0	RTX-5 SIL-MS	He	75°C(hold1min) to 300°C at 30°C min (hold 2 min)	Immunometric (f.i. ELISA, etc.)	Ion trap		
97	0.5	solving in 50 ml hot Water+1ml Acetic Acid, cooling, filling to 100ml, sonification	100	sonication	15	SPE (Cation Exchange)	20	LC	LC-MS/MS	250	2.0000000	5	Phenomenex, Aqua	A: 0,1% Acetic Acid in Water B: 0,1% Acetic Acid in Methanol	T= 30°C 0-5min 90%A/10% B, 5,5-7,5 min 20%A / 80% B, 8-15min 90%A / 10% B	MS	MS/MS		Your reporting form does not work We submit our result as scan of the reporting form and additionally as FAX
98	0.5	Diethylamine/Water/Acetonitrile (10/40/50)	10	Mechanically shaken and sonication	40	No	5	LC		100	3.000	3	Atlantis HILIC Silica (Waters)	A: Ammonium formate 10mM pH3 B: Acetonitrile	Isocratic: 5%A /95%B Isothermal: 40ºC	MS	MS-MS	Melamine 13C-15N	
99	0.3	2.5 % formic acid in DI water	3	Shaking	30	Dilute with ACN 20x, centrifuge, filter	1	LC		150	2.100	5	SeQuant, ZIC-HILIC	A = 95/5 ACN/0.1% formic acid in water B = 50/50 ACN/20mM ammonium formate in watr	100% A to 100 %B in 11 min	MS		Melamine, doubly labeled	A note on measurement uncertainty. Sample A Replicates: 10.4 and 10.4 Sample B Replicates: 3.08 and 3.16

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
100	4	acetonitrile/ TCA20% in water 50/50	80	shaking	15	SPE with SCX	150	GC		30	0.250	0	Agilent	He	100°C 1 min, 15°C/min to 250 °C, hold for 5 min, 30 °C/min to 300°C, hold 3 min	MS		cyromazine	Uncertainty in mg/kg
101	1	Diethylamine/Water/Acetonitrile (10/40/50)	20	Shaking 10' and Sonication 30'	40	Centrifugation at 3500 rpm for 5' and Filtration of supernatant by 0.45 um nylon filter disc	1	GC		30	0.250	0	Varian Factor Four VF-5MS (0.25 um)	Helium, costant flow 1 mL/min	75°C (hold 1') to 320°C at 11°C/min (hold 3')	MS	Thermo Focus DSQ	2, 6-diamino-4-chloropyrimidine (CAS 156-83-2): only for qualitative valutation of derivatization step	ref.: FDA method, v. 2.1 - "GC-MS Screen for the presence of Melamine, Ammeline, Ammelide and Cyanuric Acid"
102	0.5	Aqueous trichloroacetic acid (TCA) 1%, w/v	10	mechanical shaking	45	No, but a tenfold dilution with HPLC water was performed for minimizing ionisation suppression. Moreover, the ion-pairing reagent (TFHA) was added to the sample vial up to 12.5 mM	20	LC		50	2.100	5	Discovery C18 (Supelco)	(A) Water 0.5 mM TFHA (B) Methanol	0 min, 5%B; 3 min, 5%B; 6 min, 50%B; 7 min, 50%B; 8 min, 95%B; 8.1 min, 5%B; 14 min, 5%B	MS	MS/MS in SRM mode. Quantification: 127>85 and Confirmation: 127>68	Yes. 13C3 15N3 melamine (Cambridge Isotope Lab)	Melamine concentration calculated from average from six independent analysis: Milk powder: 10.3, 10.7, 11.7, 10.5, 10.7, 10.7 Baking Mix: 3.2, 3.2, 3.3, 3.3, 3.4, 3.1 Apparent recovery calculated from average from five QCs: 2.5 mg/kg (n=2), 5 mg/kg (n=2) and 10 mg/kg (n=1)

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
103	0.5	Acetonitrile/ Water/Diethylamine (50/40/10)	20	vortex + sonication	30	No	200	GC		30	0.250		Restek (RTX-5MS w/INTEGRA guard)	Helium	75°C 1 min 75-320 15°C/min 320°C 2.67 min	MS		No	
104	1	5 % (W/V) trichloroacetic acid in water	20	vortex, sonification and shaking (30 min at 60 °C) subsequently	55	no	5	LC	More specific: UPLC	100	2.100	2	Acquity UPLC BEH C18 (100 x 2.1 mm; 1.7 µm part. size)	Mobile phase A: 2.0 mmol/l HFBA in water Mobile phase B: 2.0 mmol/l HFBA in methanol	t=0-2 min: 97.5 % A and 2.5 % B t=2-4 min: 10.0 % A and 90.0 % B t=4-4.1 min: 97.5 % A and 2.5 % B	MS	More specific: MS/MS	3 C13 - 3 N15 - melamine	Standard addition is used for quantification. Internal standard is added to the sample extract in order to monitor and correct for possible ion suppression effects. Found [MEL] in samples very high! Questions from our customers mainly resulted in quantification in the range of 50 ppb to 1 ppm.
105	1	2.5 % HCOOH in water	14	Vortex	30	SPE Strata X - C wash with MeOH Elution with 5 % NH4OH/MeOH	1000	LC	UPLC	100	2.100	2	Waters Acquity UPLC BEH Hilic	90 : 10 Acetonitrile : 2 mM NH4-Formate	Isocratic	MS	LC-MS-MS		
106	1	acetonitrile water 50/50	20	shaking/sonication	30	No	1000	LC	ACQUITY UPLC with BEH HILIC	100	2.100	2	Waters	Acetonitril/10 mM Ammonium Acetate	ISOCRATIC	MS		MELAMINE 13C15N	Detection method is MSMS in MRM mode with 3 transitions. Please note that the results are not correceted for recoveries.

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
107	2	Methanol/Water/Formic acid 49/49/2	20	sonication	30	solid-phase extraction on Strata-X-C 60 mg / 3 ml; 0,5 ml sample load; 1.wash 1 ml of extraction solvent; 2.wash 1 ml Methanol; elute with 1.5 ml Methanol/Ammoniahydroxide (1,5%); blow down o dryness; Derivatisation with Pyridine/Sylon BFT	1	GC		30	0.25	0	DB5-MS	Helium 5.0	75°C, 1min. - 30°C/min - 300°C, 2 min.	MS	EI-MS	C13-Melamine	

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108	0.1	Acetonitrile / water (80:20)	13	shaking	2	Centrifugation followed by a cleanup on SPE SCX Lichrolut® (40-63 µm) 3 mL / 500 mg Merck. Conditionning with acidic water and acidic methanol; washing with water and methanol;elution with ammoniacal methanol	500	LC		150	2.100	5	Merck - SeQuant ZIC-HILIC	A: ammonium acetate 10 mM in water B: acetonitrile	0-7 min: 5%A; 7-10 min: 80%A;10-11 min:80%A;11-11.5 min: 5%A; 11.5-26.5min 5%A.	MS	MS/MS	yes: [13C3, 15N3] - melamine	- test portion size is 0.1 g for sample A and 0.5 g for sample B. - expanded uncertainties are given in % - results have not been corrected with the recoveries
109	0.5	Acetonitrile/ water 85/15	25	Shaking	1	SPE MCX cartridge (6cc) Elution with 5 mL NH4OH 5% (20/80 NHçOH 25%/water) . Derivatization of the resulting analytes with BSTFA:TMCS 99:1	200	GC		30	0.250	0	HP-5 ms Nr 19091S-433	Helium	70°c hold1 min; 25°C/min-->150°C; 3°C/min-->190°C; 50°C/min-->320°C hold 10 min	MS	single quad detector	Melamine (13C3, 99 %; Amino-15N3, 98 %)	Expanded uncertainties are given in % Results have not been corrected with the given recovery values

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110	1	Methanol/PBS 20 mM (10:90,v/v)	8	shaking	10	Protein precipitation using Carrez salts	100	None								Immuno metric (f.i. ELISA, etc.)	ELISA Abraxis - 450 nm	no	- Expanded uncertainties were not calculated considering the semi-quantitative aspect of the described procedure - Results given have not been corrected with the recovery data
111	1	5 mL Water + 5 mL 2% TCA:Acetonitrile (1:9)	10	Shaking	1	An aliquot of sample extract through a cation exchange SPE cartridge, wash with water and methanol. Elute with 1.25% ammonia in methanol. Evaporate and redissolve in formic acid:acetonitrile.	1000	LC		250	4.600	5	Supelco Discovery C18	33 mM octanesulfonic acid in phosphate buffer : acetonitrile, 85:15 (v/v)	Isocratic	UV	Photodiode Array - Wavelength 240 nm	No	- Reported results have not been corrected for apparent recovery. - Expanded recoveries are given in %

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
112	0.2	water/acetonitrile/1.0M HCl (12:12:1)	1	vortex	20	Solid phase extraction with Waters MCX 1c.c cartridge Condition: 1 mL Methanol and 1 mL DI water Loading : supernatant Wash: 1 mL 2.5 % formic acid and 1 mL acetonitrile Elute : 5 % ammonia in acetonitrile	150	Other	Capillary electrophoresis (with sweeping)	650	0.050	0	Polymicro Technologies (Phoenix, AZ, USA)	50 mM H3PO4, 175 mM SDS	25 degree Celsius	UV		none	
113	1	Extraction solvent acetonitrile: water, 50:50	20	Shaking on automated wrist action shaker	20	Yes. Sample extract cleaned up with Waters OASIS MCX columns.	2000	LC		150	2.1	5	SeQuant ZIC-HILIC	A - 5% 10mM ammonim acetate in 95% acetonitrile; B - 10mM ammonium acetate in 50:50 acetonitrile:water	Gradient: A - 0-10 mins for elution; B - 10.1 - 15 for wash; A - 15.1 - 30 for equilibration	MS	MS/MS	Isotopically labelled melamine standard (13C) with molecular weight of 129. Ratio of native transition (127 --> 85) to internal standard transistion (129--> 87) were used for calibration curve and quantitation. Native transition (127-->68) used for confirmation of	No baking mix was used as negative control. A sample of melamine free milk powder was used as a control The spike recoveries were 101% and 106% respectively. A method blank was also run. There was no contamination present. Spiking level was 1 mg/kg. The uncertainty is based on the method uncertainty.

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
																		identity.	
114	0.5	Acetonitrile/ Water/Diethylamine (50/40/10)	20	Vortexing and sonication	50	No	200	GC		300	0.250		Restek-5MS	Helium (1.0 mL / min.)	100 C (1.5min)->15degrees/min->150 C (0.0)->7 degrees/min->200 C (0.0)-> 15 degrees/min-> 320 C (5.0 min.)	MS		Yes. 4-Chloro-2,6 diamino-pyrimidine (DACP)	This method for GCMS is a "semi-quantitative method"
115	0.5	Acetonitrile / water (50/50),	10	blending and sonication,	40	yes , SPE-SCX	500	GC		30	0.200		Supelco SPB1	He	1 min at 75 °C, rate 15°C/min to 300°C per 5 min	MS		benzoguanamine	
116	0.5	50:50 ACN:water	10	vortex and mechnical shaking	15	yes, Oasis MCX SPE	30	LC		150	2.100	5	Waters Atlantis HILIC	90:10 A:B A= 5% water 95% ACN 5mu ammonium acetate B= 1:1 ACN :water 10 mu ammonium acetate	isocratic during elution; gradient at end of run to clean column	MS		yes, 13C, 15N melamine	
117	0.5	10:40:50 DEA :water:acetonitrile	20	shaking and sonication	45	filtration of a portion of supernatant through 0.45 um nylon filter disc	700	GC		30	0.250	0	Agilent	Helium	initial temp 100 C ; temp program (I):100-210 C rate 10 C/min; temp program (II): 210 -300 C rate 30C/min runtime=25 minutes	MS		DACP	

Lab ID	Smp weight [g]	Extraction solvent	Volume extr. solv. [mL]	Agitation	Dura-tion [min]	Clean-up	Volume test solution [µL]	Sepa-ration	Sepa-ration if other	Column length [m or mm]	Col. ID [mm]	Col. particle size [µm]	Col. brand	Mobile Phase	Gradient	Detection	Detection if other	ISTD	Remarks
118	0	2.5% (v/v) Formic Acid	0	Sonication and wrist-action shaker	0	Filter using a 0.22 micron membrane microfilter	0	LC	LC-MS/MS	150	2.100	5	Sequant ZIC-HILIC	Mobile phase A: 95:5 ACN: 0.1% Formic Acid Mobile phase B: 1:1 ACN: 20 mM Ammonium Formate	See FDA LIB 4421 for detail (attached)	MS		None	
119	0.5	DEA/ Water/ Acetonitrile (10/40/50)	20	Vortex + Sonication	30	No	500	GC		30	0.250	0	J and W Scientific	Helium	75-280 degrees at 15 degrees C per minute after 1 minute initial hold and 5.33 minute final hold	MS		2,6-Diamino-4-chloropyrimidine	
120	1	Acetonitrile / water (50/50)	20	sonicate 5 min., vortex 20 min.	25	Yes, Waters Oasis MCX for melamine, MAX for cyanuric acid.	10	LC	LC in HILIC mode	150	2.100	5	SeQuant	phase A: 5% 10mM ammonium acetate : 95% ACN phase B: 10mM ammonium acetate in 50:50 ACN:Water	100% phase A, followed by washout with phase B after analyte elution, then return to 100% phase A before next injection	MS	positive ESI Triple quadrupole MS (negative ESI for cyanuric acid)	15N3 - Melamine and 15N3 13C3 - Cyanuric Acid	Method is United States FDA LIB 4422 Measurement of uncertainty above is in mg/Kg

European Commission

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Title: **Melamine Proficiency Test 2009 – Assessing the capabilities of control laboratories to measure melamine in skimmed milk powder and starch-containing foods.**
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Abstract
A proficiency test to assess the capabilities of laboratories in the EU and beyond to determine melamine in a milk powder and a baking mix, representing starch-containing foods like bread and biscuits, was carried out in January of 2009 by the Joint Research Centre upon request of the European Commission's Directorate-General for Health and Consumer Protection (DG SANCO). The need for such an interlaboratory comparison arose from a health scare in China about melamine tainted powdered milk in the second half of 2008.

Laboratories of 31 countries, including Australia, China, India, Japan, New Zealand, the United States of America, and 21 of the 27 Member States of the European Union, participated and reported back 114 results for the milk powder and 112 for the baking mix test materials. The reported results were compared to reference values determined by exact-matching double isotope dilution mass spectrometry. The so determined assigned values were 10.0±0.6 mg/kg melamine in the milk powder and 3.18±0.17 mg/kg melamine in the baking mix. A coverage factor k of 2 was applied to calculate the expanded uncertainties.

Three-quarters of all reported results for both materials had associated z-scores which were satisfactory ($z \leq |2|$). 90% of the results were accompanied by a measurement uncertainty statement and the majority of the measurement uncertainty ranges were reasonable. A number of laboratories were found to underestimate their measurement uncertainties.

Isotope dilution mass spectrometry with stable-isotope labelled melamine was shown to be clearly advantageous with regards to the accuracy of the results. However, no significant influence by other method parameters could be identified.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

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